ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

1965-1966

DIVISION OF BIOLOGICS STANDARDS
DIVISION OF COMPUTER RESEARCH AND TECHNOLOGY
DIVISION OF RESEARCH FACILITIES AND RESOURCES
DIVISION OF RESEARCH GRANTS
DIVISION OF RESEARCH SERVICES

NATIONAL INSTITUTES OF HEALTH
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Annual Report of Program Activities

July 1, 1965 through June 30, 1966

DIVISION OF BIOLOGICS STANDARDS

ANNUAL REPORT

July 1, 1965 through June 30, 1966

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DIVISION OF BIOLOGICS STANDARDS

Summary of the Director

The National Institutes of Health and its precedent organizations have had the responsibility, since 1902, for the control of biological products. Early in the history of these institutions, the regulatory function was carried out by a relatively small group of scientists, administered as a "division" or "laboratory" depending on the organizational structure. These scientists performed their own research on vaccines, serums, and antiserums, blood and blood derivatives, etc., and their control activities, within the same environment as the other scientists in the small enclave of the National Hygienic Institute or on the large campus of the National Institutes of Health. Collaboration within the small Institute was the rule, and the regulatory function was carried out with the aid of scientists not directly involved in it. Conversely, the research activities of the staff involved in control work contributed to the vigor of the entire organization.

Ten years ago, in recognition of the advances being made with vaccines, of the burgeoning of virology, and of the growing complexity of immunology and hematology, the Division of Biologics Standards was established as a part of the National Institutes of Health with a status equivalent to that of other Divisions and Institutes. Division scientists still profit from collaboration with colleagues in other Institutes and still contribute to the intellectual vigor of the NIH.

This is highly salutary. It is, indeed, hard to imagine how the regulatory functions of the Public Health Service Act could be carried out except in such a climate. The Division is always confronted with problems that involve requirements for the most sophisticated data of modern virology, oncology, and molecular biology. We cannot forget "old" products such as smallpox and yellow fever vaccines; and we must be continually cognizant of advances in microbiological and pharmaceutical research which affect the standards and the techniques of immunization. We must also be prepared to fill gaps in our knowledge of immunization against old and new agents, to set standards for relatively rarely used but highly important materials such as antivenins and antitoxins; and we must strive to maintain high standards in the distribution of blood and the separation and purification of blood products. The expertise involved in all these functions can be provided only in a vigorous research environment.

While the research of the Division is directed to a large extent towards these goals, we would indeed be remiss in meeting our responsibilities if we did nothing more. We cannot be a completely passive agency, waiting for results obtained by other organizations, either public or private, that we can apply. The Division must be aware of the moving front of biomedical research, not only to be prepared for new demands for standards of safety, purity, and potency of biologics being developed for use in the prevention or treatment of human disease, but also to single out and investigate new problems gouged out by the moving glacier of advancing knowledge.

As an example of the last point, the Division has the responsibility for investigating the relation of recently described avian leucosis agents, found in eggs, to the safety of yellow fever vaccines, which have been produced in chick embryos for many years. While the chances appear small that such agents have a deleterious effect on human beings vaccinated with attenuated yellow fever virus grown in chick embryos, we would be derelict in our duty did we not approach the problem from a control and an investigative standpoint. We thus become involved in research of both a fundamental and practical nature.

Other examples are the elucidation and identification of covert viruses in tissue cultures which may be used in the manufacture of vaccines, the investigation of chemical and physical factors involved in the inactivation of vaccines, and the study of the ingredients added to injectable products purported to make them more effective for longer periods. With certain agents, such as influenza, which vary widely in successive epidemics, the Division staff must keep constantly alert to the epidemic intelligence provided by health agencies around the world, so as to be able to recommend new formulations of virus strains in vaccines for each season.

These functions, obviously, cannot be carried out by an organization dedicated solely to the routine examination of products destined for human use. They involve far more: Constant attention to developments in the fields of microbiology, hematology, allergology, and immunology, and research directed towards the problems which arise with new information concerning biological products.

This year's report reflects the diverse nature of the work of the Division. Those functions of the Laboratory of Control Activities which in former years were reported as research projects are now summarized as control work. The index, however, will show that scientists in this Laboratory are also active in research collaboratively with personnel in the other Laboratories of the Division. Similarly, the control and service activities in other Laboratories, previously described as projects, are now included in the Laboratory Chiefs' summaries. There remain to be reported as research projects a wide range of studies bearing on many factors involved in the development and control of effective and safe biologics.

Scope of Control Testing Program

Since the individual Laboratory reports, when reviewed separately, do not reflect the magnitude of the control testing program which extends through the entire Division, summary tables have been prepared in an attempt to show the scale of the Division-wide effort. These data represent only one phase of the control of biological products.

Essentially the control testing program operates in the following manner: Manufacturers of a licensed product submit to the Division samples from each lot of the product together with protocols containing the results from all tests performed on that lot. Some 294 different biological products are currently licensed. Samples and protocols are submitted on approximately 175 of these.

Each protocol and sample is reviewed by the Division to determine if the manufacturer's test results conform with established standards. In addition, some or all of the tests required on a particular product may be performed by the Division. If the manufacturer's test results and the results of tests performed by the Division conform with standards, a letter of release for that lot is sent to the manufacturer. Only then may the manufacturer distribute this particular lot for sale.

During the 12-month period covered by this report, 3,438 lots of biologic products were released, 66 lots were rejected and 55 lots were withdrawn by the manufacturer. Table 1 reflects the number of lots released by categories of products.

Table 1

Product Group	Lots Released	Lots Rejected	Lots Withdrawn	Totals
Antitoxins	40	0	1	41
Therapeutic Serums	20	0	1	21
Blood and Blood Products	855	2	7	864
Bacterial Vaccines	113	1	3	117
Toxoids and Toxins	74	0	0	74
Multiple Antigen Preparations	139	7	6	152
Viral and Rickettsial Vaccines	785	31	13	829
Diagnostic Substances for Dermal Tests	199	0	0	199
Diagnostic Substances for Laboratory Tests	1,066	25	24	1,115
Miscellaneous	147	0	0	147
Totals	3,438	66	55	3,559

A protocol may consist of considerable information requiring up to 200 pages, for example, on a lot of Poliovirus Vaccine, Live, Oral, or very succinct data on only two pages for a lot of blood grouping serum. All protocols are recorded and reviewed by members of the Laboratory of Control Activities. The protocols are then forwarded to other Laboratories within the Division for review; the nature of the product determines the Laboratory(s) consulted. For example, a protocol for Measles Virus Vaccine, Live, Attenuated, is reviewed by personnel in six of the Division's Laboratories.

Some of the Division personnel are involved full-time in the control program, while many other individuals are involved on a much smaller scale. As the control testing on products becomes more specialized, a larger number of the research scientists are becoming involved in control activities.

Table 2 shows the total number of control tests performed by Division personnel last year on the 3,559 lots submitted for release. This gives no idea of the total effort involved, however, since tests requiring 15 minutes or less are given the same weight as those taking a month or more to complete. Continual attention is given to test development, and progress on these endeavors is to be found in various of the project reports of the different Laboratories. It is in this area, especially, that staff of the Laboratory of Control Activities are working in collaboration with other Division scientists.

Table 2

Total Control Tests Performed in Reporting Period

Type of Test		Number of Tests
Potency	In Vivo	1,737
	In Vitro	11,942
Safety	General	1,040
	Specific	4,851
Purity		10,740
Sterility		5,845
Total Control Tests		36,155

The control activities of the Division require, also, the maintenance of a large number of different standard and reference materials. These are prepared in the Division or obtained by purchase. They are all tested in the Laboratories of DBS and distributed as needed. During fiscal 1966, over 7000 such preparations were sent out.

This illustrates another facet of the Division, the close relationship which has been built up between manufacturers and the regulatory agency facilitates the maintenance of high standards for biological products needed to protect the health of the community. In this respect, the Division again does more than await developments made elsewhere. The industry looks to the Division for the guidelines necessary for improving the quality and maintaining the safety of its products.

In support of its control activities, which require the yearly inspection of establishments as well as the review of protocols and the testing of products, the Division carries on an inspector-training program. New staff members from the various Laboratories have participated in a series of seminars held during the year. These sessions deal with the responsibilities and authorities of the Division as defined in the Public Health Service Act, with licensing

requirements for establishments, and with criteria to be used on inspections. In addition, Division personnel participate in an inspector training course sponsored by the Division in conjunction with the Food and Drug Administration and the University of Rhode Island.

Licensing and Investigations

The Licensing and Investigations Section of the Office of the Director, Division of Biologics Standards, has as its principle responsibility the correlation of enforcement activities arising from the Division's responsibilities under the Public Health Service Act, 42 U.S.C. 262. The following statistics summarize the activities of the units for Licensing, Inspections, and Investigations, from July 1, 1965.

LICENSING:

Licensing actions are based on the recommendations of license review committees appointed by the Director from the scientific staff of the Laboratories concerned with the particular kind of product or establishment.

As of April 1, 1966, 212 establishment licenses, with 80 subsidiary establishments, were in effect. Between July 1, 1965, and April 1, 1966, 10 establishment licenses were issued and eight revoked without prejudice. Two establishment licenses were revoked for cause. These were Dallas Blood Bank, Inc., Dallas, Texas, license No. 353, and Metro Blood Service, Inc., of Philadelphia, Pennsylvania, license No. 352. During the first nine months of fiscal year 1966, 37 product licenses were issued, 19 revoked without prejudice and four revoked for cause. One thousand three hundred twenty-two product licenses are in effect for 297 different licensed products.

Thirty-one applications for an establishment license, 66 for product licenses and 24 to amend effective product licenses were pending at the time of this report.

INSPECTIONS:

Thirty-four members of the scientific staff, selected by Laboratory Chiefs for their special knowledge and authorized as inspectors by the Secretary, Health, Education, and Welfare, perform the inspections which are planned and coordinated by the Office of the Director. Seventeen additional professional employees assist in the inspection program.

As of April 1, 1966, 48 of 60 scheduled inspection trips for the fiscal year 1966 were completed. During these trips, 169 licensed establishments, 93 locations, 107 blood donor centers and 6 applicants for an establishment license were inspected, for a total of 375 inspections. Approximately 30 different products were examined.

To accomplish these inspections, 350 work days were required with 495 man work days, as some inspections were performed by two inspectors.

Summary

Scheduled trips performed	48
Licensed establishments	169
Locations	93
Donor centers	107
Pre-Licensing	6
Total visits	375
Work days	350
Man work days	495

INVESTIGATIONS:

Investigations, in contrast with licensing and inspections, are conducted by the inspectors in the Office of the Director with assistance from laboratory staff when needed for peak loads. Inspections call for the expenditure of predictable periods of time on the part of the people involved and can thus be carried out with minimum dislocation of their other duties. Investigations, on the other hand, are essentially "open ended" with respect to time and must therefore be carried out by people who have this duty as their main function.

As of April 15, 1966, the legal actions and investigations of possible violations of the Public Health Service Act, involved a total of 142 man work days on travel status. One hundred seven man work days were devoted to consultations with state and federal officials, criminal trial preparation and participation, and witness interviews. The remaining 35 man work days on travel status were spent visiting both licensed and unlicensed establishments for enforcement purposes.

During the period ending May 1, 1966, the following criminal actions resulted from Division of Biologics Standards investigations:

1. One case went to trial and the defendants were found guilty on each of 26 counts. One defendant was sentenced to serve four years in prison, the other defendant to serve 60 days with three years' probation.

2. A plea of "nolo contendere" was made in one case with subsequent fines imposed.

3. A 14 count Information was filed in one case.

4. A plea of "guilty" to two out of 14 counts was made in one case with subsequent fines imposed.

5. In one case a plea of "guilty" to all counts was made by the corporation with subsequent fines imposed.

6. A defendant in an earlier case was denied his petition to the U. S. Supreme Court for a Writ of Certiorari.

Contract Operations

The Division has utilized contracts on a relatively small scale for several years, for such purposes as the development of sources of snake venoms, improvement of standards of potency of blood-typing sera, and the performance of studies on the hemagglutination level of pooled human plasma. These have been summarized in past annual reports. In fiscal 1966, the Division received an additional appropriation from the Congress, together with the charge to investigate problems of oncogenesis associated with viral vaccines and other products.

To organize this activity and to coordinate the contractual work with the scientific program carried out within the Division, a new assistant director was appointed at the beginning of January, 1966. He is Dr. Leon Jacobs, formerly Acting Scientific Director of the National Institute of Allergy and Infectious Diseases.

Procedures for the initiation of contractual projects and for the dual review of contracts have been set up. A great deal of effort has been expended in obtaining information on contractual operations of other Institutes or Divisions within NIH that bear on the questions of viral and chemical carcinogenesis. The DBS Contracts Committee, consisting of the Assistant Director, the Laboratory Chiefs, and a few other people, has been apprised of this information. We have, therefore, gained the necessary background to orient our own efforts in directions that are especially important to the Division's mission. Although the section on contracts appended to this report does not yet reflect this progress, because the contracts are not yet formally let, we have developed a sizeable program. A contract for the testing, in baby hamsters, of the oncogenetic potentialities of a variety of viral vaccines or possible "candidate" vaccines, is being established at the time of this writing. Other contracts for the procurement of intermediate products in vaccine preparation are in the process of development. These materials will be tested in the baby hamster system, probably with the establishment of another contract. Still additional projects on the chemistry, toxicology, pharmacology, and oncogenicity of various inactivants, stabilizers, and preservatives used in vaccine manufacture, and of mineral oils and emulsifiers proposed for use as adjuvants in allergenic products and vaccines, have been advertised to potential contractors.

It is noteworthy that the development of this contract program, which is now gaining momentum, has proceeded in what we consider an ideal way. Contractual operations should be, properly, extensions of the activities of the Division staff. The contract on baby hamster testing has been developed as the result of considerable activity on the part of Dr. Amos Palmer of the Laboratory of Pathology and Mrs. Claire L. Cox, recently assigned from the Laboratory of Bacterial Products to the contracts program. These scientists, under the supervision of Dr. Ruth L. Kirschstein, Chief of the Laboratory of Pathology, have been engaged for two months in preparatory work on product-testing in baby hamsters, on the development of coding systems, and the procurement of products. Because of their efforts, we are now prepared for the initiation of operations by the contractor and for the proper supervision of his activities. Another example of the participation of staff in the contract effort is the

determination of what additional materials, for tests in hamsters, will be produced within the Division or procured from outside sources. As a result of special meetings, Division personnel have established the need for outside procurement of only 50 per cent of needed materials; the rest will be furnished by staff members who are especially competent to produce them. An additional example can be cited: The DBS Contracts Committee was interested in the development and testing of yellow fever vaccine freed of avian leucosis agents. As the result of committee meetings held by Dr. Nicola Tauraso, the appointed project officer, it has developed that there is enough interest and enthusiasm among many staff members to provide for the performance of most of the necessary work within the Division; only a small fraction, if any, will have to be done by contract.

This, indeed, signalizes the value of scientific program coordination. We expect, confidently, that the contractual operations will continue to be closely related to the activities of Division staff. Some contracts which were in effect for other purposes have been re-oriented to fit into the oncogenesis testing program. For example, the University of Texas contract on plastics-biologics interaction is being oriented, beyond toxicology, to the study of oncogenic effects. This is the result of the interest of Division staff members in certain observations noted by the contractor.

Office of the Director

Program direction and administration for the Division of Biologics Standards is based in the Office of the Director, with centralization of administrative procedures relating to personnel, travel, supply, and accounting. Major objectives are determined and program responsibilities are assigned to the operating units within the Division. For each major area, staffing patterns are developed and are continually re-evaluated in relation to program changes. The required support is determined for each area in relation to the Division's total budget. A major activity of the Director's office is the maintenance of the proper balance between control activities and research programs, and as stated above, coordination of contractual and "in-house" activities.

The Office of the Director includes an Information Office, a Biometrics Section, an Experimental Virology Section, an Investigational New Drug Office, and a Scientific Communications Office.

The Director uses his Information Office for communications to the public of significant aspects of the Division's research and control activities, and for reports to higher echelons within the Public Health Service and the Department, and to the Congress.

The Biometrics Section is set up within the Director's Office because its functions cut across the entire Division. In the report of this Section, it will be noted that some of its activities are designated as "tasks". These represent the performance of work, using already established statistical techniques, for solving problems related to vaccine-testing originating in various Laboratories. In addition, the Section is involved in research on perfecting

statistical methods to serve the needs of the Division. This involves the problems of acceptance sampling and contingency table analysis, relating respectively to the laboratory examination of vaccines for safety and to the evaluation of field trials of candidate vaccines.

The "IND" Office, set up to examine "Notices of Claimed Investigational Exemption for a New Drug", which are required by section 130.3 of the Food and Drug Regulations, has continued to function within the Office of the Director. During the period covered by this report, 35 notices and 205 supplements were received and studied. The Office has carried on an extensive correspondence to correct deficiencies in submissions and to point out hazards. The notices add to the Division's alertness regarding the development of new products. The staff has opportunity to "tool up" in advance for the formulation of regulations and standards for such new products.

Data and information pertaining to licensed biological products and to new products in the offing are of immediate and direct importance to the regulatory and control programs. The Office of Scientific Communications performs very valuable functions for the Division in its bibliographic and reference activities. It maintains current awareness procedures designed to retrieve selected types of information for Division staff. It is also working on the concept of more effective and rapid means of information processing. As it is evident that the entire field of biomedical research requires new information retrieval techniques, such developments, for DBS, should proceed along with systems that are being worked out for the whole body of this knowledge. This requires us to maintain close contact with information specialists working elsewhere within NIH and in other agencies.

The program of the Experimental Virology Section will be more properly included in the summary of research activities.

Acquisition of Building 29-A

Shortage of space in all categories continues to be a factor which restricts full implementation of the functions of the Division in many areas. At the close of fiscal year 1966 the Division was occupying some office space in building 12-A, cold room space in buildings 5 and 13, and animal space in building 14-D. In addition, the assembly-lunch-room in building 29 was being used as office space. The problems raised by the shortage of space should be resolved when the new building (29-A) is completed. The building, which has been in course of planning, design, and construction since 1962, should be completed by mid-November 1966. Construction has been carried out on schedule and the completion date seems realistic. The main loss of time was a delay of approximately one year during the architectural and engineering design stage.

Acquisition of the additional space should make it possible to consolidate within buildings 29 and 29-A all of the activities of the Division with the exception of some animal space in building 14-D.

Personnel

Mr. John M. Easton, who performed some valuable studies on virus "hybridization" with Dr. Caspar W. Hiatt in the Laboratory of Biophysics and Biochemistry, transferred in July, 1965, to the National Cancer Institute to undertake a residency in pathology. His place was filled, in the same Laboratory, by Dr. Kendall O. Smith, who is exploiting advanced virological and electron microscopic techniques for the study and characterization of adeno-associated viruses and other agents.

Notable recognition of the competence of Division personnel was the assignment of Dr. Harry M. Meyer, Chief, Laboratory of Viral Immunology, to accompany Secretary John W. Gardner on his mission to Viet Nam. Dr. Meyer was a member of the team headed by PHS Surgeon General Stewart, to investigate what health measures could be applied in South Viet Nam. Dr. Meyer was chosen because of his previous experience with mass measles vaccination campaigns in West Africa.

Dr. Meyer and Dr. Paul D. Parkman of his Laboratory were honored by the Public Health Service and the Department in April for their development of an experimental attenuated rubella virus strain which may serve for the preparation of vaccines. Dr. Meyer received the PHS Meritorious Service Medal and Dr. Parkman a Superior Service Award.

Many other members of the Division have been recognized by invitations to serve on WHO panels and special committees, and by nominations to serve as leaders of symposia at scientific meetings. These recognitions indicate the high regard in which the Division scientific staff are held by their peers.

Research and Development

The following summaries of the programs of the Laboratories present in more detail the research and development activities of the Division. As has already been mentioned in regard to the research work of personnel of the Laboratory of Control Activities, there is considerable inter-Laboratory collaboration within the Division. For example, the rubella studies in the Laboratory of Viral Immunology involved also Dr. Ruth L. Kirschstein, Chief of the Laboratory of Pathology and Mrs. Hope Hopps of the Laboratory of Virology and Rickettsiology. The work now to be described will be, for convenience, identified only with the Laboratory of the principal investigator. However, the individual project reports will identify the other individuals and groups who have contributed to it.

Section on Experimental Virology

The continuing investigations of this Section relate especially to the question of viral oncogenesis, and to the circumstances under which tumor-formation occurs or can be suppressed by oncogenic viruses.

Following reports that certain human adenoviruses were oncogenic for hamsters, adenovirus type 12 and simian virus 40 were used in studies to determine the possibility of preventing tumors in hamsters by means of vaccines. Repeated

large doses of homologous type virus given to hamsters which had been infected neonatally either with adenovirus type 12 or simian virus 40 resulted in suppression of tumor-production. Many hamsters, and in some experiments all the animals, failed to produce tumors. Inactivated homologous virus failed to produce this effect.

Suppression of tumor-production occurred also when simian virus 40 was inoculated into newborn hamsters and adenovirus 12 was injected in repeated doses later. Here an interesting phenomenon was observed: Two strains of adenovirus 12 were used. These are indistinguishable by many tests, such as virus neutralization and cross protection. However, they were found to differ in their tumor-suppressing capacity in hamsters that had been infected with simian virus 40 when newborn. The strain which exerted the protective effect had a history of having been propagated in monkey kidney epithelium cultures where it could have been in contact with simian virus 40. The strain which was not protective had been isolated and propagated in human cell cultures and never in monkey kidney cell cultures. Simian virus 40 could not be recovered from the protective strain; and according to tests carried out in the Laboratory of Infectious Diseases, NIAID, no simian virus 40 or tumor antigen could be demonstrated either by complement fixation or by immunofluorescence.

This program is of major significance in developing a full understanding of hazards that may exist in proposed live virus vaccines and in providing information on the importance of the immune state in relation to viral oncogenesis.

LABORATORY OF BACTERIAL PRODUCTS

Cholera Vaccine and Related Studies. Field trials of potency of cholera vaccines in East Pakistan showed significant protection lasting for as long as two years. However, in the Philippines, no protection was apparent after less than six months except when the vaccine was used with an oil-in-water adjuvant. Abscesses following administration of this type of adjuvant preclude further use, but aluminum adjuvants may be able to effect longer persistence of immunity without such side effects.

Recent results suggest that cholera in the suckling rabbit may be due to toxin (choleragen or Craig's skin toxin). It appears that the passive protection of the infant rabbit described in last year's report may have been due to antitoxin. Because of the current interest in cholera toxins, studies are being directed towards detoxification, assay of toxin and toxoid, and the role of the toxoid in assay procedures.

Laboratory personnel are heavily engaged in the cooperative work on cholera being sponsored by WHO, the Pakistan-SEATO Cholera Research Laboratory, and the U.S.-Japan Cooperative Medical Science Program. The Chief, Laboratory of Bacterial Products, Dr. Margaret Pittman, has continued to serve as the NIH Project Officer of the Pakistan SEATO laboratory and as a member of several advisory committees. Dr. John C. Feeley spent two months as a travelling consultant for WHO reviewing potency trials. Dr. Feeley is also a member of the U.S. Panel on Cholera of the U.S.-Japan Cooperative Medical Science Program.

Pertussis vaccine. Two candidate preparations of pertussis vaccine have been characterized and tested in comparison with the Japanese toxicity reference and vaccines used in a clinical trial in Great Britain. The toxicity assay results obtained by the collaborators, with one strain of mouse, differentiated the two vaccines according to a clinical reactivity which was significantly different. In these tests a common strain of mouse was used. Our study indicates that different strains of mice, and conventional versus germ-free mice differ significantly in toxic reactions to pertussis vaccine. These factors will be considered in the clinical study now in the formative stage and in the specifications for freedom-from-toxicity testing of pertussis vaccine.

In order to determine the antigenic pattern of U.S. vaccine production strains of Bordetella pertussis, and of strains isolated from occasional cases of whooping cough, and to have typing antisera produced for reference purposes, a contract was initiated in June 1966 with the Michigan State Department of Health. This will provide needed information on changes in antigenic pattern of B. pertussis in the United States, to compare with reports from England and Canada.

Tetanus Toxoids. In the cooperative study on the prevention of neonatal tetanus in the Territory of New Guinea, the results indicate that if women of child-bearing age were vaccinated against tetanus and given a booster injection of either plain or adsorbed tetanus toxoid, they would respond with antitoxin titer levels sufficient to protect the newborn child. Adsorbed

toxoid induced higher titers and would be preferable for booster as well as for primary immunization as previously reported.

The study on the assay of the unitage of the adsorbed and plain toxoids used in the field trials indicates that the animal-measured unitage was correlated with duration of antitoxin levels. The two types of toxoids differed about 6-fold in unitage. Although primary response to each toxoid was similar, at the end of two years the mean antitoxin level of the women who received the adsorbed toxoid was about 6-fold greater than of those who received plain toxoid.

The investigation of the cause of abscess-formation following the injection of two lots of tetanus toxoid in water-in-oil adjuvants is showing promising results which indicate that certain toxoid preparations (as well as cholera vaccine which also caused a high incidence of abscesses in the Philippines) and other antigens are capable of causing Arlacel A, the emulsifier in the adjuvant, to break down into free fatty acids. Oleic acid, an impurity in Arlacel A, was incriminated some years ago as the cause of abscess-formation following injection of influenza virus vaccine. It now appears that even with purified Arlacel A certain antigens will cause release of fatty acids from the emulsifier. Analyses of the free fatty acids released and their effect on tissue are being made.

Pleuropneumonia-like Organisms and Mycoplasma. A transitional L-form of Streptococcus sanguis has been isolated from the lesions of 21 patients with recurrent aphthae and from lesions of only one of six non-aphthae patients. Aphthae patients also gave a positive skin reaction to a polysaccharide extracted from the streptococcus. These results suggest a causal relation of S. snaguis to recurrent aphthae.

The Mycoplasma orale isolated by direct culture procedures from the blood and bone marrow of 4 of 10 leukemic patients and not from 10 non-leukemic patients has stimulated much interest on the possible etiological or associated role of Mycoplasma in leukemia.

Tuberculin. The significant work on the separation of tuberculin into protein and carbohydrate fractions, each of which is capable of eliciting delayed reactions in sensitive guinea pigs, has been extended to show that these fractions in Freund's Adjuvant are capable of inducing passive cutaneous ananphylaxid antibodies, also complement-fixing and precipitating antibodies. An interesting observation has been made to the effect that high concentrations of Old Tuberculin tend to suppress the response to lower concentrations of material when injected in parallel positions on opposite sides of sensitized guinea pigs. This has implications in regard to potency tests. It has been shown that, at concentrations suggested by WHO for potency testing of the International Standard, the reaction to the U.S. Standard Old Tuberculin was suppressed.

Poison Ivy. The finding obtained in one contract project that urushiol was present only in extract of freshly collected leaves and that the commercial poison ivy extracts used in another contract project did not contrain urushiol opens the need for an investigation on whether or not such extracts would be

capable of desensitizing poison ivy sensitive persons. The clinical contract project results indicate that the degraded catechols of urushiol do not desensitize.

LABORATORY OF BIOPHYSICS AND BIOCHEMISTRY

Microbial Biophysics. Theoretical investigations of problems dealing with mechanisms and kinetics of viral inactivation were facilitated by a contractual arrangement with Electronic Associates Inc. of Rockville, Maryland, which provided analog computer rental and engineering services. A set of differential equations was devised to express the survival ratio of photosensitized T_2 coliphage as a function of time, light intensity, oxygen tension, and three parametric rate constants representing the inactivation rate, O_2-diffusion rate, and rate of utilization of O_2 within the phage particle. The computer was used to solve these equations simultaneously and to plot any selected variable as a function of time. The solutions allowed the selection of critical conditions for laboratory experiments. It was found that oxygen concentration in the photosensitized phage particle decreases as it is used up by the photochemical reaction and as it diffuses into the medium. For significant photodynamic inactivation (90% or more) to occur, the velocity constant for diffusion must be less than 10% of the velocity constant for inactivation.

Photodynamic inactivation curves for T_3 coliphage sensitized with toluidine blue and irradiated under a variety of conditions were found to fit closely to theoretical curves for a model in which each of four critical sites must be destroyed in order to kill the phage.

Theoretical curves on the effects of aggregation on the inactivation of virus suspensions were calculated from published data on distribution of aggregates of various sizes in vaccinia virus preparations. Apparent velocity constants for inactivation of highly aggregated preparations are about 25% less than the values for monodisperse suspensions of single particles.

These studies on the mode of action of viral inactivating processes are of continued value to the Division in providing fundamental information for the control of viral vaccines.

Analytical Chemistry. An original method for measuring mercurial preservatives in biological products, employing the atomic absorption spectrophotometer to determine the amount of mercury, has been developed to the point where it can now be evaluated on various vaccines. The spectrophotometer has also been used to detect an easily measurable amount of thallium in an experimental Mycoplasma vaccine purported to be thallium-free. Analytical methods for phenol in the presence of high concentrations of protein, and for benzethonium chloride, have been perfected. An electrolytic apparatus to measure moisture in dried biologics has been put into service to be evaluated in relation to conventional methods.

Virus characterization. Adenovirus 4 particles were found to have a density of 1.34 gm/cm^3 in cesium chloride. A small DNA virus, designated adeno-associated virus or AAV, symbiotically associated with one of several

adenovirus 4 strains investigated, has been shown to have a density range overlapping adenovirus 4. Separation of adenovirus completely free of AAV by density gradient centrifugation would therefore be impossible. However, since some AAV particles have densities ranging up to 1.44 gm/cm^3, it is possible to separate AAV completely free of adenovirus by centrifugation in cesium chloride. Preparations of AAV thus purified have been used to prepare antisera against AAV, and these antisera have provided means to rid adenovirus of AAV. The diameter (18 mμ - 21 mμ) and fine structure of AAV have been determind; it appears to be the simplest possible icosahedron, having a reovirus-type net-like capsid. AAV is structurally unrelated to its associated adenovirus. AAV is more resistant to detergents than adenovirus.

AAV-antibody reactions have been observed in the electron microscope in a manner which permits visualizing the attachment of gamma globulin molecules upon the viral surface. AAV appears to be antigenically distinct from adenoviruses in neutralization, agglutination, and complement fixation tests, although both grow to high concentrations in the same cell cultures. Measurable replication of AAV was sought in cell culture systems, but was never observed in the absence of adenovirus. It is therefore concluded that AAV requires adenovirus as a helper.

The studies are especially relevant to the production of viral vaccines, in which the occasional presence of adventitious agents has constituted a serious control problem. The capability of detecting contaminating viruses by direct physical observation, and promptly and definitively characterizing new viruses is regared as an important asset to the DBS program.

LABORATORY OF BLOOD AND BLOOD PRODUCTS

The research program of this Laboratory has included studies of the stability of blood products, the development of methods and standards for these products, the investigation of red cell antigens, plasma antibodies, and the clotting and fibrinolytic systems, and the examination of proteins of body fluids. These various fields of interest have as their goal the improvement of procedures used for the control of safety, purity and potency of biological products derived from blood. Thus the projects are directed toward improving existing control tests, developing new ones, obtaining stability data leading to more realistic dating periods, and providing the professional staff with constantly updated information necessary in evaluating new products and procedures.

Results obtained to date indicate that the amount of trichloroacetic acid-soluble material in immune serum globulin increases progressively during storage. Concomitantly, material with a sedimentation coefficient of approximately 3S appears in these preparations, and the amount of such material increases with time. Starch gel electrophoresis has demonstrated an increase in the number of protein components during the storage period.

To develop methods for measuring the physical and chemical interaction of a perservative and surface antiseptic with a plastic which may be used as a packaging material for biologicals, a study of the pilot system, benzalkonium chloride + nylon-6,6, has been undertaken. Measurements made

thus far appear to confirm the initial hypothesis that the diffusion, permeation, and solubility coefficients depend on concentration, temperature, and time. Other factors that must be considered are the cationic characteristics of benzalkonium and its critical micelle concentration.

To evaluate the effect of high molecular weight additives on blood typing serums, various concentrations of polyvinylpyrrolidone (PVP) have been added, in the presence and absence of bovine serum albumin (BSA), to systems containing group O, Rh_o (D) positive cells and anti-Rh antiserum. It was found that a thousand-fold increase in titer (compared with controls containing no PVP but equivalent levels of BSA) could occur when agglutination was carried out in the presence of 1% PVP and 5% BSA. PVP in the absence of albumin caused no rise in titer.

Basic research in the Laboratory has included investigations on various phases of the clotting and fibrinolytic systems and studies of the proteins of body fluids.

When fibrinogen rich in antihemophilic factor (AHF) was clotted by thrombin, most of the AHF activity was lost. Similarly, even in the absence of active thrombin (i.e., after inactivation of the thrombin with diisopropylfluorophosphate) polymerization of fibrin by calcium was accompanied by significant losses of AHF. By addition of 0.3 M glycine ethyl ester to the clotting medium, however, it was shown that in the presence of both calcium and active thrombin 80 to 90 per cent of the fibrinogen could be clotted with a loss of less than 10 per cent of the total AHF activity.

Investigation of an increase in antihemophilic factor activity in the plasma of mice bearing plasma cell tumors was performed. Of 20 lines of mouse plasma cell tumors tested, the only line that caused an increase plasma level of antihemophilic factor also caused the accumulation of a macroglobulin in the plasma. This macroglobulin was therefore isolated and injected into mice without tumors. Subsequent to the injection there was a 2-fold increase in antihemophilic factor activity which lasted approximately 48 hours; appropriate controls showed no increase.

LABORATORY OF PATHOLOGY

The Laboratory of Pathology has now completed its first full year as a separate entity. Until this year, the Laboratory's largest project has been the performance of neurovirulence tests of lots of Poliovirus Vaccine, Live, Oral. However, with the great decline in poliomyelitis in the United States and in the world, the number of lots submitted for testing has markedly decreased. As a result, the Laboratory has been able to turn its attention to other problems related to the pathogenesis of infectious diseases and their prevention or control. In addition to its own research and control activities, the Laboratory has had an active collaborative research program with members of the staffs of other Division Laboratories and with members of other Institutes as well.

Neurovirulence Testing. Several Type III Live Poliovirus vaccines were tested repeatedly and the reproducibility of the test system could be

demonstrated. The data presented led to development of an international study involving the comparative testing of vaccines of varying degrees of neurovirulence. This study is now in progress.

Secondary and primary seed lots of Yellow Fever Vaccine have been studied in rhesus monkeys so that, in the event that a new seed lot is needed, the neurovirulence level of the vaccine will have been established.

Oncogenesis and pathogenesis of infectious agents. Studies on the comparative oncogenic effects of polyoma virus strains, simian virus 40 and adenoviruses have been continued. It has been found that polyoma virus produces tumors only in mice, hamsters and mastomys, SV40 in hamsters and mastomys, adenoviruses in hamsters, mastomys and certain inbred strains of mice. The oncogenic potential of these viruses can be enhanced by decreasing the immunologic capability of these animals; this can be accomplished primarily by performing thymectomies on newborn animals. In addition, studies of a "hybrid" virus of adenovirus 7 and SV40 (strain E46) indicate that the genome of either the adenovirus portion or the SV40 portion have specific oncogenic effects under varying conditions. Studies on the pathogenesis of tumors produced by clones of various SV40 plaque sizes are of different virulence and oncogenic potential.

Studies on cells in vitro, either derived from tumors, or "transformed" by oncogenic viruses, have been performed. The cellular transformations have characteristics of malignancies and have varying growth potentials when inoculated into animals, depending on the species.

Studies of the pathologic reaction in muscles of monkeys injected with tetanus toxoid emulsified in mineral oil adjuvant reveal that the emulsion tends to localize in pockets throughout the muscle and to cause severe reactions in the muscle as long as 6 months after inoculation. It appears that this material may remain in the muscle forever and continue to act as an irritant. Studies of other adjuvants are planned so that information will be available when they are proposed for use.

The Laboratory has assumed a major responsibility for a contract on the testing of various vaccines and viruses for oncogenesis. This contract has just been let, but staff personnel have spent several months determining proper dosages of materials for baby hamsters and in organizing the program.

Neuro-anatomy. Studies on the neuro-anatomical development of various animal species have provided the baseline information for work on the immunologic and allergic response of the central nervous system to infection or immunization.

LABORATORY OF VIRAL IMMUNOLOGY

Rubella. A major research emphasis of the Laboratory has been development and clinical testing of an attenuated rubella virus suitable for use as an immunizing agent. This work was initiated in order to "tool up" the competence of the Division in anticipation of the need to test candidate rubella vaccines. However, as a result of tissue culture studies on the

rubella virus, it became apparent that attenuation could occur in high passage levels.

In vitro and in vivo techniques were devised capable of signaling if and when modification of cell culture-propagated rubella virus had occurred. High passage levels of the virus were found to exhibit marked differences in behavior when compared to virulent low passage virus in RK_{13} continuous rabbit kidney tissue cultures. Of even more interest was the observation that the high passage strain evoked the production of increased amounts of interferon in various types of cell cultures.

The rhesus monkey was used to compare the characteristics of infections produced by the virulent and the modified high passage virus. These animals served as an ideal experimental model since the virologic events associated with virulent rubella virus infections in monkeys closely simulated the circumstances observed in man. The tests with the high passage strain provided the final clue to the fact that laboratory manipulation had, indeed, attenuated the virus. The animals inoculated with virulent rubella virus developed infections characterized by viremia, virus shedding from respiratory and rectal secretions, and communicability to uninoculated cage contacts. Monkeys infected with the high passage strain developed antibodies but did not exhibit viremia or significant virus shedding, and uninoculated controls remained free of infection.

The high passage strain was used to prepare an experimental live rubella virus vaccine. This material was inoculated into 8 rubella-susceptible girls under carefully supervised conditions of isolation. None of these children showed any symptoms of illness which could be attributed to the vaccine, yet all developed neutralizing antibodies indicative of immunity. Viremia was not detected. Although small amounts of virus were obtainable from the pharynx of several vaccinees, the material was not communicable, since none of 8 rubella-susceptible children were inoculated and maintained in close association with 22 susceptible contacts. Again there was no clinical disease and no evidence of communicability.

This work has major scientific import for the following three reasons: (1) It provides the first evidence that rubella virus can be attenuated by laboratory manipulation; (2) it provides the first "marker" techniques that are capable of distinguishing between virulent and attenuated rubella viruses short of clinical trial and (3) it provides an attenuated strain of the virus that is promising for further investigation in the search for a vaccine.

Viral Genetics. In other investigations this Section again turned to the highly sensitive qualitative complement-fixation techniques which in the past year's annual report had been shown capable of detecting subtle antigenic differences between closely related viruses. The current work with the technique has demonstrated that the Freund and Rauscher mouse leukemia viruses appear to be antigenically identical.

Mumps. Two new procedures have been developed for detecting mumps virus antibodies. Both of these methods (a modified hemagglutination-inhibition test and a plaque-reduction neutralization test) are more sensitive than the

conventionally employed antibody assay techniques. These new procedures were developed in anticipation of future needs in research and control obligations concerned with the prevention of mumps.

Vaccinia. Rhesus monkeys have been used to study the cutaneous and immunologic response to inoculation with vaccinia virus. Interest has been focused on determining the importance of the route of inoculation and virus dose on immunity. Animals have been vaccinated by (1) the multiple pressure technique, (2) jet inoculation, (3) intradermal inoculation, and (4) subcutaneous injection. Inocula have contained from 10^2 to 10^8 $TCID_{50}$ of vaccinia virus.

By varying the virus dose and route of inoculation, it was possible to evoke antibody production without eliciting a dermal response. This simulated the type of human response observed during clinical investigations with dilutions of smallpox vaccine given by jet injection.

LABORATORY OF VIROLOGY AND RICKETTSIOLOGY

Arboviruses. The newly established arbovirus collection has been greatly expanded to include other viral (and mycoplasma) antigens and antibody reagents. This was a result of the decision to establish an immunoserology unit to serve as a nucleus for a major service and research component of LVR. At the time of this writing (April), 184 agents are in the working stock of 300 frozen lots of "master", "working", and "vaccine" virus pools, 112 antigens for complement fixation and hemagglutination-inhibition, and 122 antibody reagents (hyper-immune sera and ascitic fluids). Very recently 65 new arbovirus prototypes were received from Yale Arbovirus Unit; master and working pools will be produced shortly. Techniques of immunodiffusion and agar gel diffusion have been added to the already routinely performed quantitative complement fixation, hemagglutination-inhibition and immunofluorescence techniques.

A new study on the electromicroscopic morphology of certain viruses was undertaken in collaboration with Dr. K. O. Smith, LBB. An early result was the determination of the myxovirus nature of the Kemerovo virus prototype (isolated by Soviet workers from ticks and humans). The agent was definitively identified as Newcastle disease virus by J. Casals and ourselves. Human epidemics due to Kemerovo virus have presented a serious public health problem in Siberia.

Avian leukosis. Owing to the Division's increasing responsibilities related to avian leukosis viruses, a major build-up of the required facilities has been accomplished during the year. The combination of RIF tests in tissue culture, serological COFAL tests, and immunofluorescence covers the gamut of techniques necessary to cope not only with the potential RIF contamination of egg-produced vaccines, but prepares us for the future should the virus of Marek's disease of fowl prove to be a tumor virus potentially hazardous to humans receiving egg-produced vaccines. (A reference collection of candidate Marek's virus agents is being set up.) Such experience will serve as a base for other oncogenic virus work.

Four Rous sarcoma virus tumors (Bryan, Zilber-Carr, Prague and Armstrong) have been maintained in hamsters by transplantation; recently the tumor cells of all four were successfully adapted to tissue culture opening up a new area for research and development. A new and important method for determining fowl maternal RIF antibody (or virus) in egg yolk by immunofluorescence has been worked out. This should allow ready monitoring of chicken flocks and preparation of RIF-free cell cultures from chick embryos taken from eggs with yolk known to be negative by this specific immunofluorescence test.

Adenoviruses. Current studies on adenovirueses serve to accumulate information which will be of help in determining the safety of candidate adenovirus vaccines for use in man, and, in addition, will be of assistance in understanding the biology of adenovirus infection. During the past year, the early findings of Huebner and associates on the "hybridization" of adenovirus type 7 and SV40 were extended by showing that SV40 genetic material is apparently also enclosed within adenovirus type 3 capsids. Related to this are current studies on the occurrence of SV40 antibody in the sera of people vaccinated with the adenovirus lots known to contain SV40 genetic material and on the possible serological response to the "adeno-associated virus" particles known to be present in many adenovirus vaccines.

Influenza. The Section on Respiratory Viruses continues to develop information which should be of assistance in improving the effectiveness of influenza virus vaccines. For instance, evidence has accumulated to indicate a marked recent shift in the antigenic composition of some of the contemporary strains of influenza B. This affects the Division's plans for the construction of the next vaccine formula.

Important data on the relationship of serum neutralizing influenza antibody levels and resistance to subsequent influenza virus challenge were obtained from a human volunteer study. Even more important information on the dynamics of continual change in the antigenic composition of influenza viruses may be obtained from a field study of immunologically virginal populations of several Pacific atolls. Some of the island groups experienced a severe epidemic of influenza in 1964; their antibody responses to epidemic infection are being followed and the response to monovalent vaccine will be examined. An additional group of nearly 1,000 people, without influenza A or B antibody, who had escaped infection during the 1964 epidemic, offers a unique opportunity to evaluate immunological responses to monovalent influenza virus vaccines.

Rickettsiae. Studies have concerned interferon production, parasite-host-cell relationships, comparative electronmicroscopic morphology, and genetic relatedness of rickettsiae, mycoplasmas, and viruses. Interferon production does not seem to be a prominent feature of rickettsial proliferation in vivo. On the other hand, the level of interferon production may serve as a reliable marker for rubella virus attenuation. Careful collaborative electromicroscopic studies of mycoplasmas, larger viruses and rickettsiae showed that the 3 groups of organisms are morphologically distinguishable from one another.

Studies on the characteristics of virulent and avirulent rickettsiae

(typhus, spotted fever and scrub typhus) are continuing because of the need for a better understanding of the phenomenon of attenuation in relation to live rickettsial vaccines. Certain fractions of the killed typhus rickettsiae (soluble antigen, cell wall and intracellular protoplasma) are being compared with the intact organism to define their relative immunogenicity as a step toward more potent, purified vaccine. As a refinement rickettsiae grown in cell culture rather than in eggs are now being tried. Initial steps toward developing a vaccine potency test using monkeys, rather than guinea pigs, have been taken by determining the susceptibility of rhesus and cercopithecus monkeys to epidemic typhus rickettsiae.

The Laboratory now possesses a competency to perform studies on the genetic relatedness of rickettsiae using the DNA homology techniques. The initial studies employed the DNA from R. quintana; the technique will be applied to other rickettsiae, mycoplasmas and probably viruses.

Tissue culture. In an effort to establish a back-up cell line for BS-C-1, particularly as needed for measles vaccine testing, a second line of cercopithecus kidney cells has been under test - this is designated BS-C-2 (MA-134) cell line. Extensive comparative studies indicate that BS-C-2 can satisfactorily replace the standard BS-C-1 line.

The Tissue Culture Section continues to supply the entire Division with with large numbers of primary and continuous cell cultures from verifiable sources and of high quality. Some 400,000 cell cultures in tubes and 150,000 in bottles of different sizes were issued during 1965. This service activity occupied practically the entire time of the Section's personnel.

However, new emphasis on research and development has resulted in the introduction of several new methods into production; these have improved the quality and quantity of cell cultures at sizable savings. New research projects deal with studies on malignant cell transformation in vitro, using human diploid cells (WI-38 cells), which are of particular concern to DBS, having been proposed for use in human vaccines. This possibility has stimulated another project, the purpose of which is to determine the presence of blood group or tissue transplantation antigen in cultured WI-38 cells, because of a potential hazard of sensitization of vaccine recipients.

Paolins. Studies of the antitumor effects of clam extracts, presumably containing the active principle (paolins), have been extended to include adenovirus 12 tumors (virus-induced or transplanted) in hamsters, mouse leukemia (L-1210), mouse sarcoma 180, and mouse melanoma (S-91). Preventive treatment of newborn hamsters soon after inoculation with oncogenic adenovirus 12 seemed to inhibit tumorformation. Weaned hamsters with transplanted solid adenovirus 12 tumors shed the tumors and survived indefinitely when the clam extract was injected into and around each tumor. Mice with otherwise lethal melanomas, leukemias and sarcomas responded to clam extract treatment, sometimes in a most dramatic fashion. To allow general confirmation and extension of these challenging results, a large quantity of clam extract has been prepared by a commercial laboratory on contract with the Division. The material will be used to supply the demands of other investigators at NIH and elsewhere and for further attempts at characterization, isolation, and

identification of the active principle in clam extract.

Hepatitis. The A-1 agent described some years ago as a virus recovered from an icterogenic human plasma pool, has now been definitely identified as a strain of Mycoplasma gallisepticum. Serological tests, by plaque reduction and growth inhibition techniques, of sera from various series of human cases of hepatitis, indicate some relation of this Mycoplasma to the disease. However, more work is necessary to rule out anamnestic responses in the human cases tested and to be sure that significance can be attached to small rises in titer. Moreover, it has not been possible to re-isolate the Mycoplasma from the icterogenic pool. The new approach to the A-1 agent suggests a large number of additional studies.

Serial No. 01
1. Experimental Virology
2. Office of the Director
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Experimental Virology

Previous Serial Number: Same

Principal Investigator: Bernice E. Eddy, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	3
Professional:	1
Other:	2

Project Description:

Objectives: To investigate the safety of biological products, especially in relation to the tumorigenicity of viruses and means of preventing tumor-production; investigate new viruses and other agents in relation to their importance in human disease and for the purpose of exploring the possibility of preventing diseases that at present are uncontrolled.

Methods Employed: Conventional methods of inoculation of viruses grown in various tissue culture systems of serological determination of antigenic relationships, and of pathologic examination of tissues.

Major Findings: Following reports that certain human adenoviruses were oncogenic for hamsters, adenovirus type 12 and simian virus 40 were used in studies to determine the possibility of preventing tumors in hamsters by means of vaccines. Repeated large doses of homologous type virus given to hamsters which had been infected neonatally either with adenovirus type 12 or simian virus 40 resulted in suppression of tumor-production. Many hamsters, and in some experiments all the animals, failed to produce tumors. Inactivated homologous virus failed to produce this effect.

Suppression of tumor-production occurred also when simian virus 40 was inoculated into newborn hamsters and adenovirus 12 was injected in repeated doses later. Here an interesting phenomenon was observed: Two strains of adenovirus 12 were used. These are indistinguishable by many tests, such as

virus neutralization and cross protection. However, they were found to differ in their tumor-suppressing capacity in hamsters that had been infected with simian virus 40 when newborn. The strain which exerted the protective effect had a history of having been propagated in monkey kidney epithelium cultures where it could have been in contact with simian virus 40. The strain which was not protective had been isolated and propagated in human cell cultures and never in monkey kidney cell cultures. Simian virus 40 could not be recovered from the protective strain; and according to tests carried out in the Laboratory of Infectious Diseases, NIAID, no SV40 or tumor antigen could be demonstrated either by complement fixation or by immunofluorescence.

Significance to Bio-medical Research and the Program of the Division: This program is of major significance in developing a full understanding of hazards that may exist in proposed live virus vaccines and in providing information on the importance of the immune state in relation to viral oncogenesis.

Proposed Course of Project: Work will be continued on immunologic effects in relation to tumorigenic activity of various viruses, employing techniques and experimental protocols similar to those above but varied in different parameters.

(With Dr. A. Morris and others) A study of a possible hybridization of adenovirus type 20 and simian virus 40.

(With Dr. C. P. Li and others) Studies concerning the antiviral or antitumor effect of clam extracts on hamster tumors induced by adenovirus type 12 or simian virus 40.

(With Dr. Michael Barile) A study of the effect of Mycoplasma on the oncogenic effect of certain viruses.

(With Dr. Kendall O. Smith) A study involving antigens of some of the adenoviruses.

Honors and Awards: None

Publications:

Eddy, B. E.; Young, R. D.; and Grubbs, G. E. Method for inhibiting oncogenesis in hamsters infected when newborn with simian virus 40. Perspectives in Virology. 4: 209-217 (Editor, Morris Pollard, Hoebner Medical Division of Harper and Rowe Publishers, Inc., New York, 1965)

Li, C. P.; Prescott, B.; Eddy, B. E.; Caldes, G.; Green, W. R.; Martino, E. C.; and Young, A. M. Antiviral activity of paolins from clams. Ann. New York Acad. Sciences. 130: 374-382, 1965.

Eddy, B. E. Repeated injection of hamsters with oncogenic viruses as related to tumor development. I Congress Centro-americano y 11 Nacional de Microbiologia, San Jose, Costa Rica. 1: 93, 1965.

virus concentration (100% inhibition of normal cell metabolism at challenge virus concentration), rubella sera of known antibody content were tested to accumulate data on the reproducibility of the test. The results of 14 tests indicated that the inhibition of RK_{13} cell metabolism was not rapid enough to permit the practical determination of endpoints. Variation in titers between different tests ranged between 4 and 8-fold. Recently the RK-T-17 line has been tested to establish optimal cell and virus concentrations. Further tests are planned to investigate the reproducibility of rubella antibody levels in tests using these cells.

3) A complement-fixation (CF) test utilizing a standard Bengston technique with commercial antigens was developed. A number of sera from children with clinical rubella were tested for CF antibody in acute and convalescent phase paired specimens. As reported previously the CF antibody apparently rises later and to a lower level than neutralizing antibody. Sera from adults without a history of recent rubella infection show little or no CF activity when tested at a 1:8 dilution.

4) In continuing studies of rubella immunofluorescence, an indirect technique has been developed using RK_{13} cells and anti-rabbit or anti-human gamma globulin fluorescein conjugate. RK_{13} cells infected with rubella virus have reliably shown a granular pattern of intracytoplasmic antigen after 4 days of incubation when stained by this procedure. The technique however, still does not appear sufficiently sensitive or reliable for routine detection of rubella antibody. The procedure may be useful in other studies currently in progress for detection of rubella virus in other types of culture and tissues.

Project Description: IV. Studies of the antigenicity of inactivated preparations of rubella virus.

No work in this phase during reporting period.

Project Description: V. Studies of possible rubella virus oncogenic effects.

Although to date no evidence has been produced suggesting an oncogenic role for rubella virus, experimental studies in suckling hamsters have been continued. Earlier experiments (DBS 49 Pt.V) failed to suggest that low passage level rubella virus produced oncogenic effects in hamsters. To determine the effect of serial passage in GMK cultures, 60 baby hamsters less than 24 hours of age were inoculated with the 77th passage rubella virus pool (HPV-77). These animals have been observed daily for evidence of illness and development of palpable tumors. No symptoms have developed in inoculated animals. None of the surviving hamsters have developed tumors during the 7 months since inoculation. There is no evidence to date that rubella virus induces tumors in this animal species.

Project Description: VI. Studies of rubella virus infection in experimental animals.

During the past year studies in experimental animals have been primarily directed toward elucidation of the behavior of low and high rubella virus passage levels (see above). Work is currently in progress on studies of high passage rubella virus infections in pregnant rhesus monkeys. These studies are being performed according to procedures which have been reported from this laboratory. Virologic data are not yet avaliable; however, no adverse effects have been observed on the course of the pregnancies or fetuses observed thus far.

Significance to Bio-medical Research and the Program of the Division:

All work pertains to problems concerned with the prevention of rubella and the control of the rubella vaccine.

Honors and Awards: Dr. Parkman--

Superior Service Award, Dept. of Health, Education, and Welfare, April, 1966.

Letter of Commendation from President of the United States, Lyndon B. Johnson, May 5, 1966.

Dr. Meyer--

Meritorious Service Medal of the Department of Health, Education, and Welfare, United States Public Health Service, 1966.

Letter of Commendation from President of the United States, Lyndon B. Johnson, May 5, 1966.

Publications:

Parkman, P. D., Phillips, P. E., Kirschstein, R. L. and Meyer, H. M., Jr. Experimental Rubella Virus Infection in the Rhesus Monkey, J. Immunol. 95: 743-752, 1965.

Parkman, P. D., Phillips, P. E. and Meyer, H. M., Jr.: Experimental Rubella Virus Infection in Pregnant Monkeys, Amer. J. Dis. Child. 110: 390-394, 1965.

commercial laboratory on contract with the Division. The material will be used to supply the demands of other investigators at NIH and elsewhere and for further attempts at characterization, isolation, and identification of the active principle in clam extract. (Rep. No.74,p.182).

Hepatitis. The A-1 agent described some years ago as a virus recovered from an icterogenic human plasma pool, has now been definitely identified as a strain of Mycoplasma gallisepticum. Serological tests, by plaque reduction and growth inhibition techniques, of sera from various series of human cases of hepatitis, indicate some relation of this Mycoplasma to the disease. However, it has not been possible to re-isolate the Mycoplasma from the icterogenic pool. Also, more work is necessary to rule out anamnestic responses in the human cases tested and to be sure that significance can be attached to small rises in titer. The new approach to the A-1 agent suggests a large number of additional studies.

CONTRACT ACTIVITIES

A new contract (under No. PH-43-66-35) was made with Melpar, Inc. to prepare a large quantity of clam extract so that it can be made available for study at LVR and elsewhere. Some 250 gallons of shucked fresh clams (Mercenaria mercenaria) were purchased, frozen and are being gradually processed.

The initial interest in antiviral effects of the extract has gradually shifted to a concern with its antitumor properties. Accordingly, the techniques of extract preparation and of its assay for specific activity have been modified (see Dr. Li's Individual Project Report No. 74, p.182). Limited attempts at purification and concentration were made at Melpar by such means as Sephadex fractionation and high speed centrifugation followed by DEAE treatment.

SERVICE AND CONTROL FUNCTIONS

Most of the working hours in the Tissue Culture Section are spent on the purely service function of providing the entire Division with cell cultures of research quality from verifiable sources. In addition, some time is spent on a control activity of safety testing the inactivated virus vaccines in cell culture.

During the calendar year 1965 the following cultures were prepared by the Section - 398,150 tubes, 129,532 2-ounce bottles, 2,102 milk dilution bottles and 4,988 32-ounce bottles of both primary and serial cell cultures. Sizable quantities of rhesus, cercopithecus, and BS-C-1 cell cultures were used in the safety testing of live poliovirus and live measles vaccines. Large quantities of cercopithecus, RK-13 and MA-104 cell cultures were used in the rubella program. More than 27,000 ml of cell suspension were prepared from 9 serial cell lines to investigate the activity of clam extracts in vitro. Several lots of primary cells of rat, dog and guinea pig kidneys, mouse

embryo, monkey heart, monkey testes and monkey ovaries were processed on request from interested investigators. Production records of primary and serial cell cultures are summarized in Tables 1, 2 and 3.

The Respiratory Virus Section is responsible for potency testing of influenza and adenovirus vaccines. One hundred and eleven influenza virus vaccine lots were received in the laboratory of which 99 were manufactured for civilian use and 12 - for the military. Seventy-nine of the civilian vaccine lots were examined on the basis of the manufacturers' protocols only, 17 lots were selected for testing by DBS, and 3 lots were withdrawn by the manufacturer. Of the 12 military vaccines, 2 were withdrawn by the manufacturer and the other 10 were tested in our laboratory; the manufacturers' protocols were examined in all cases. (No adenovirus vaccines were submitted for release during this year).

The Avian Leukosis Unit of the Section on Experimental Virology performed 30 RIF tests on 23 live measles vaccines, all of which were found to be negative for the RIF virus by this procedure.

No rickettsial vaccines were submitted for testing by the Laboratory during this year.

CONTRACTS

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LABORATORY OF BACTERIAL PRODUCTS (LBP)

Determination of the Potency of Poison Ivy Extracts by Clinical Studies. PH43-64-885 Orentreich Research Corporation. $23,500
Project Officer: Harold Baer, Ph.D.
Principal Investigator: Robert Auerbach, M.D. - - - - - - - - - - 16,23

Preparation of Poison Ivy Compounds. PH43-64-76 Columbia University. $122,869
Project Officer: Harold Baer, Ph.D.
Principal Investigator: Professor Charles Dawson, Ph.D. - - - - 16,23

Serological Analyses of Bordetella Pertussis Strains. PH43-66-552 Michigan Department of Health. $21,900
Project Officer: Margaret Pittman, Ph.D.
Principal Investigator: Grace Eldering, Ph.D. - - - - - - - - - - 17

LABORATORY OF BIOPHYSICS AND BIOCHEMISTRY (LBB)

Analog Computer Data. PH43-65-1052 Electronics Associates, Inc. $4,450
Project Officer: C. W. Hiatt, Ph.D.
Principal Investigator: C. W. Hiatt, Ph.D. - - - - - - - - - - - - 53

Toxicological Evaluation Reports. Professional Services Contract #246940. $2,250
Project Officer: Jerome J. Helprin, Ph.D.
Principal Investigator: W. Von Oettingen, Ph.D., M.D. - - - - - -54

LABORATORY OF BLOOD AND BLOOD PRODUCTS (LBBP)

Influence of Various Materials on Human Hemo-Antibodies. PH43-65-1046 Baylor University. $166,951
Project Officer: John N. Ashworth, Ph.D.
Principal Investigator: Sol. Haberman, Ph.D. - - - - - - - - - - - 67

Plastics-Biologics Interaction. PH43-64-557 University of Texas. $261,023
Project Officer: John N. Ashworth, Ph.D.
Principal Investigator: John Autian, Ph.D. - - - - - - - - - - - - 67

Standardization of Blood Collection Techniques. PH43-65-1053 John S. Sharpe Research Foundation. $24,748
Project Officer: John N. Ashworth, Ph.D.
Principal Investigator: Paul Strumia, M.D. - - - - - - - - - - - - 68

To Perform Studies on Hemagglutinin of Levels of Pooled Human Plasma. PH43-64-548 Southwest Blood Banks $48,609
Project Officer: Donna Williams
Principal Investigator: Marion Rymer, Ph.D. - - - - - - - - - - -

Tests Anti-Human Horse Serum by Immuno-Electrothoresis. PH43-65-98 Cordis Corporation. $21,500
Project Officer: Dr. John N. Ashworth
Principal Investigator: Dr. John Sterner - - - - - - - - - - - - -

LABORATORY OF CONTROL ACTIVITIES (LCA)
Acquire and Maintian Coral Snakes for the Extraction of Venom. PH43-65-87 Miami Serpentrarium Laboratories, Inc. $35,400.
Project Officer: Edward B. Seligmann, Ph.D.
Principal Investigator: Mr. C. E. Amory - - - - - - - - - - - - - 89

LABORATORY OF VIROLOGY AND RICKETTSIOLOGY (LVR)
Prepare, Purify, Test and Furnish Clam Extraction. PH43-66-35 Melpar, Inc. $49,100
Project Officers: Alexis Shelokov, M.D.
C. P. Li, M.D.
Principal Investigator: Mr. C. E. Amory - - - - - - - - - - - - -

SCIENTIFIC COMMUNICATIONS OFFICE (OSC)
Indexing of the WHO Working Documents from the Expert Committee on Biological Standardization. Professional Services Contract #808043. $1,200
Project Officer: A. K. Reich
Principal Investigator: Virginia Zaratzian, Ph.D. - - - - - - - - 2

BIOMETRICS SECTION
To Provide Services in the Field of Methodology Statistics, PH43-65-81 Booz-Allen Applied Research, Inc. $46,586
Project Officer: Clifford J. Maloney, Ph.D.
Principal Investigator: Mr. John Reardon - - - - - - - - - - - - - 4

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DCRT
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LBP
LBB
LBBP
LCA
LP
LVI
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INVESTIGATORS
PUBLICATIONS

Annual Report of the
Laboratory of Bacterial Products
Division of Biologics Standards
July 1, 1965 through June 30, 1966

1. Research Activities.

Cholera vaccine and related studies. (Proj. Nos. 22, 23, 24, 25, 26, 105; p. nos. 41, 44, 47, 49). Emphasis was continued on potency assay of vaccines used in field trials. The ranking of the vaccines relative to potency and efficacy agree in general. Only in East Pakistan has significant protection with reasonable duration (2 years) been obtained. In the Philippines protection was nil in less than six months except with an oil-in-water adjuvant. Abscesses following use of latter adjuvant preclude further use. In E. Pakistan the infection was due largely to Inaba serotype vibrios and in the Philippines--Ogawa serotypes. There is need to investigate the effect of aluminum adjuvants on persistence of immunity. LBP will participate in procuring and testing of adsorbed vaccines.

With the use of the infant rabbit model of cholera it appeared in last year's report that passive protection was effective only when 7S antibody was present. Recent results, however, suggest that protective activity may have been due to antitoxin and that cholera in the suckling rabbit is caused by toxin (choleragen or Craig's skin toxin). Because of the high current interest in the toxins of cholera vibrios studies are being directed towards the detoxification, assay of toxin and toxoid and the role of the toxoid in assay procedures.

Pertussis Vaccine. (Proj. No. 104, p. no. 39). Two candidate preparations of pertussis vaccine have been characterized and tested in comparison with the Japanese toxicity reference and vaccines used in a clinical trial in Great Britain. The toxicity assay results obtained by the collaborators, with one strain of mouse, differentiated the two vaccines according to clinical reactivity which was significantly different. In these tests a common strain of mouse was used. Our study indicates that different strains of mice and conventional versus germ-free mice differ significantly in toxic reactions to pertussis vaccine. These factors will be considered in the clinical study now in the formative stage and in the specifications for freedom-from-toxicity testing of pertussis vaccine.

Tetanus Toxoids. In the collaboratory study on the prevention of neonatal tetanus in the Territory of New Guinea the results indicate that women of child-bearing age vaccinated against tetanus and given a booster injection of either plain or adsorbed tetanus toxoid would respond with antitoxin titer levels sufficient to protect the newborn child. The adsorbed toxoid induced the higher titers and would be preferable for booster as well as for primary immunization as previously reported (Proj. No. 12, p. no. 27).

The study on the assay of the unitage of the adsorbed and plain field trial toxoids indicates that the animal measured unitage was correlated with

duration of antitoxin levels. The two types of toxoids differed about 6-fold in unitage. Although primary response to each toxoid was similar, at the end of two years the mean antitoxin level of the women that received the adsorbed toxoid was about 6-fold greater than of those that received plain toxoid (Proj. No. 14, p. no. 31).

The investigation on the cause of the abscess formations following the injection of two lots of tetanus toxoid in water-in-oil adjuvants is showing promising results which indicate that certain toxoid preparations as well as cholera vaccine which also caused a high incidence of abscesses in the Philippines and other antigens are capable of causing Arlacel A, the emulsifier in the adjuvant, to break down into free fatty acids. Oleic acid, an impurity in Arlacel A, was incriminated some years ago as the cause of abscess formation following injection of influenza virus vaccine. It now appears that even with purified Arlacel A certain antigens will cause release of fatty acids from the emulsifier. Analyses of the free fatty acids released and their effect on tissue are being made (Proj. No. 15, p. 33).

Pleuropneumonia-like Organisms and Mycoplasma. A transitional L-form of Streptococcus sanguis has been isolated from the lesions of 21 patients with recurrent aphthae and from lesions of only one of six non-aphthae patients. Aphthae patients also gave a positive skin reaction to a polysaccharide extracted from the streptococcus. These results suggest a causal relation of S. sanguis to recurrent aphthae.

The Mycoplasma orale isolated by direct culture procedures from the blood and bone marrow of 4 of 10 leukemic patients and not from 10 non-leukemic patients has stimulated much interest on the possible etiological role of Mycoplasma in leukemia.

Tuberculin. The significant work on the separation of tuberculin into protein and carbohydrate fractions each of which is capable of eliciting delayed reactions in sensitive guinea pigs has been extended to show that these fractions in Freund's Adjuvant are capable of inducing passive cutaneous anaphylaxis antibodies, also complement fixing and precipitating antibodies. From a large batch of unheated tuberculin extension of the work will follow (Proj. No. 100, p. no. 18).

Poison Ivy. (Proj. No. 102, p. no. 23) The finding obtained in one contract project that urushiol was present only in extract of freshly collected leaves and that the commercial poison ivy extracts used in another contract project did not contain urushiol opens the need for an investigation on whether or not urushiol should be a specified constituent of poison ivy extract and whether or not such extracts would be capable of desensitizing poison ivy sensitive persons. The clinical contract project results indicate that the degraded catechols of urushiol do not desensitize.

2. Control Activities

(A) Standards. In collaboration with the Laboratory of Control Activities technical specifications for Diphtheria Toxin for the Schick Test and for Typhoid Vaccine were prepared and submitted to licensed

manufacturers for comment prior to finalization of Additional Standards for the products; and specifications were prepared for procurement of new U.S reference preparations for the two serotype antigens of cholera vaccine. Progress was made toward finalization of Additional Standards: Pertussis Vaccine for publication in the Federal Register. A proposed potency assay for poison ivy extract was formulated and distributed to manufacturers.

(B) Potency and safety tests. (January 1, 1965 to December 31, 1965) The Laboratory of Bacterial Products performed all the potency and toxicity tests for pertussis vaccine and the potency tests for pertussis and H. influenzae sera. The tests that were performed are listed in the table below. Besides the tests performed for release, inspection and license application purposes, a number of courtesy tests were performed on request from foreign countries. The cholera vaccine tests were performed largely relative to efficacy in man. Some of the courtesy pertussis vaccine tests were related to correlation of laboratory assay results with reactivity in children and others to aid foreign countries in improving their product.

Control tests and protocol reviews

Type of product	Purpose	Potency	Safety Toxicity	Protocols reviewed
Pertussis Vaccine	Official*	168	169	89
Pertussis Vaccine	Special, foreign	41	10	
Cholera Vaccine	Coop. LCA	16		23
Cholera Vaccine	Special, foreign	30		
Typhoid Vaccine	Special		5	
Pertussis Immune Serum Globulin	Special	6		
Anti-Hemophilus influenzae serum type b	Official	6		1
Haemophilus influenzae typing serum	Official	1		6
BCG Vaccine	Official	5		
			PPLO	
Measles Vaccine	Official		22	104
Rubella Vaccine	Special		2	2
Staphylococcus Vaccine	Special	69		
Totals		342	208	225

* Tests of samples for release, inspection or license application.

(C) Activities requiring travel status.

Type of activity	Personnel	Locations	Man days of travel
Annual Inspections	5	25	33
Investigations	1	1	1
Contracts	1	2	3

(D) License applications and amendments.

Reviews initiated	28	Personnel participation	55
Labeling reviews	39		

(E) Other activities. Three foreign visitors spent one to three weeks in the Laboratory studying control procedures.

The Chief, LBP, has continued to serve as NIH Project Officer for the Pakistan-SEATO Cholera Research Laboratory (CRL), as a member of the NIH Cholera Advisory Committee and other advisory committees. The CRL is making noteworthy contributions in the pathogenesis, epidemiology, therapy and immunology of cholera.

Dr. Feeley spent two months as a consultant for WHO around the world reviewing potency assays of cholera vaccines used in field trials.

One staff member participated in two Round Table Conferences in Europe organized by the International Biological Standardization Committee, another participated in two domestic conferences and gave two lectures and one paper and others gave lectures and papers.

3. Collaborative (Contract) Activities.

The two contracts pertaining to poison ivy extract were continued and one pertaining to pertussis vaccine was initiated at the end of the year. With each project, the project officer actively participated. Summaries of the projects follow:

(1) Preparation of poison ivy compounds. (Proj. No. 102, p. 23) The contractor is Columbia University and the principal investigator is Professor Charles Dawson. The project was initiated in November 1963 and $122,869 has been allocated. The termination date is August 1966. The results so far obtained and the prospects of capitalizing on past experience indicate that the contract should be considered for renegotiation.

Extracts from poison ivy leaves have been chemically analyzed and synthetic catechols have been prepared. The isolated and synthesized compounds have been tested for skin reactivity with use of sensitized guinea pigs. The relation of the compounds to homologous and cross sensitization and possible use as desensitizing substances are being studied. The recent observation that extracts of freshly collected leaves have a high urushiol content while extracts of leaves a few months old have no urushiol but degredation catechols, will be of high significance in specifying quality of source material for preparation of poison ivy extract, provided it is established that urushiol is the most active ingredient in sensitization and not the degraded catechol compound. Hence, additional work is proposed.

(2) Determination of the potency of poison ivy extracts by clinical studies (Proj. No. 102, p. 23). The contractor is Orentreich Research Corp. and the principal investigator is Dr. Robert Auerbach. The project was

initiated in May 1964 and the termination date is March 1967. Total amount allotted is $23,000. The skin reactivity of commercial poison ivy extract has been determined on poison ivy sensitive persons but attempts to desensitize by injection or by oral administration of the product have failed. In view of the finding (project above) that urushiol is present in extracts of fresh leaves, and none was present in the commercial extract used in skin testing or treating patients it is indicated that further work be done to determine desensitizing activity of extracts containing urushiol. Animal experimentation by the Division will need to precede this phase.

(3) Serological analyses of Bordetella pertussis strains. (Proj. No. 104, p. 39). The contractor is Michigan Department of Health and the principal investigator is Dr. Grace Eldering. The project is for one year and approximately $22,000 was allotted. Reports from England and Canada have claimed that increase or occurrence of whooping cough in vaccinated children in those countries is due to a change in the antigenic pattern of the causative strains and that these serotype strains are not represented in the production of pertussis vaccine. The potency of the vaccine used was not taken into consideration, the experimental work has yet to be confirmed, and there has not been an increase in whooping cough in the United States. However, it seemed advisable to determine the antigenic pattern of U. S. vaccine production strains, and of strains isolated from occasional cases of whooping cough and, also, have produced typing sera for reference purposes. The work was initiated at the end of the year and no findings have been obtained.

Serial No. DBS 100
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The composition, immunology, and specificity of tuberculin

Previous Serial Numbers: DBS 2, 4, 5, 6, 7, 35, 37

Principal Investigators: Harold Baer, Ph. D.
Sotiros D. Chaparas, Ph. D.

Other Investigators: Robert W. Kolb, M. S. (LCA)
Clifford J. Maloney, Ph. D. (OD)
Ruth L. Kirschstein, M. D. (LP)
Harry T. Aylor (LCA)

Cooperating Units: Laboratory of Pathology
Laboratory of Control Activities
Office of the Director

Man Years

Total:	2.1
Professional:	1.0
Other:	1.1

Project Description:

Objectives: The overall objective is to isolate the specific skin reactive substance(s) of tuberculin, to characterize its chemical and immunological properties and to evaluate the validity of the currently used potency assay for tuberculins with a view to improving tuberculins for human skin testing and the laboratory assay.

This project is divided into a series of subprojects with current correlary objectives as follows: (1) To study methods of inducing and measuring delayed sensitivity and antibody to the carbohydrate and protein components of BCG culture filtrate; (2) to determine the nature of the lesions that arise when Freund's type adjuvants are injected into guinea pigs; (3) to review and evaluate the control testing procedures in the assay of tuberculin preparations.

Methods Employed: Guinea pigs were injected with heat-killed BCG in saline, oil, or emulsion with or without carbohydrate and protein fractions. This results in lesions at the injection site and induction of delayed sensitivity and circulating antibody. Lesions were studied histologically,

delayed sensitivity by intradermal tests and antibody by complement fixation, passive cutaneous anaphylaxis (PCA) and gel diffusion. Statistical analyses were utilized where appropriate.

Major Findings:

Subproject 1: Tuberculin reactivity and antibodies to carbohydrate and protein components of BCG culture filtrate (Harold Baer and Sotiros D. Chaparas). Carbohydrate and protein induce equally good delayed, or tuberculin, reactions in sensitive guinea pigs. Animals made sensitive with heat-killed BCG had complement-fixing antibody but no PCA antibody to either protein or carbohydrate fractions.

When either carbohydrate or protein is added to the heat-killed BCG in an emulsion, delayed sensitivity, complement-fixing, PCA, and precipitating antibodies are produced in all animals.

Subproject 2: Induction of tuberculin sensitivity in guinea pigs with fractions from BCG culture filtrates mixed in oil emulsions containing Nocardia asteroides (Sotiros D. Chaparas and Harold Baer). The initial phase of this project has been completed and the results published.

Subproject 3: Induction and mechanism of immunologic tolerance to tuberculin fractions induced in guinea pigs by large sensitizing doses (Sotiros D. Chaparas and Harold Baer). The results were prepared for publication.

Subproject 4: Pathogenesis of lesions at the sensitization site in animals receiving oil-water emulsion adjuvants (Sotiros D. Chaparas and Ruth L. Kirschstein). Hypersensitivity plays a major role in the rate of development of the lesions. Within a single day of injection of 1 mg BCG in oil in presensitized animals a significant size lump was observed, whereas it took almost a week to develop comparable size lesions in animals not presensitized. Other findings back this observation. There was also a toxic element involved inasmuch as 10 mg and 100 mg of BCG without oil gave reactions within one day. Cell and serum protein studies were complicated by apparently high inapparent infections in the animal colony.

Subproject 5: Effect of the New International Standard Old Tuberculin on the reactivity of the U.S. Standard Old Tuberculin (Harold Baer and Robert W. Kolb). It was found that high concentrations of Old Tuberculin tend to suppress the activity of lower concentrations of material when injected in parallel positions on opposite sides of sensitized guinea pigs. In addition, it was shown that at the concentrations suggested by WHO for potency testing of the International Standard the reactivity of the U.S. Standard Old Tuberculin was suppressed.

Subproject 6: Statistical evaluation of standard tuberculin tests (Robert W. Kolb, Harold Baer, and Clifford J. Maloney). For each of the three types of tuberculin preparations (Old, PPD, and Tine Test) under investigation, the analysis indicated that the eye-fit estimates of relative potency essentially agree with their corresponding least squares estimates resulting from parallel line assays.

The basic assumptions of a general parallel line assay appear to be valid for the PPD and Old Tuberculin preparations.

The experimental error, which includes the dosage-guinea pig interaction, is not of appreciable magnitude to warrant suspicion and seems well-behaved relative to time.

From the data it appears that the ±20% limit of variability for Old and PPD Tuberculin preparations and the ±50% limit for Tine Test material are liberal and might be reduced in accordance with the results presented for each preparation.

Significance to Bio-Medical Research and the Program of the Division: These studies should lead to a better knowledge of the composition and specificity of tuberculin as well as the reaction of animals to the sensitizing organism. They will also result in better procedures for routine potency testing for tuberculin.

Proposed Course of Project: These studies will be continued and enlarged.

Honors and Awards: None

Publications:

Baer, H., and Chaparas, S. D.: Delayed skin reactivity to carbohydrate and protein fractions of Mycobacterium bovis (BCG) culture filtrate in the absence and presence of serum antibody. J. Immunol. 96: 353-357, 1966.

Chaparas, S. D., and Baer, H.: Lowered delayed skin reactivity to fractions of Mycobacterium bovis (BCG) in guinea pigs sensitized with large doses of BCG. Proc. Soc. Exp. Biol. Med. 120: 260-262, 1965.

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Serial No. DBS 101
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on tuberculosis vaccines

Previous Serial Number: None

Principal Investigator: Sotiros D. Chaparas, Ph. D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To develop a suitable standard or reference preparation for tuberculosis vaccine. To improve and develop assays for counting living mycobacteria in BCG vaccine, and develop tests for characterization of the organisms and their immunogenicity.

Methods Employed: Investigations of methods for determining the number of viable organisms in tuberculosis vaccines include the use of various media and cultural procedures.

Major Findings: Some problems were encountered in attempting to duplicate a manufacturer's procedure of counting viable organisms. Triton X-100 used in diluting fluid proved to be toxic for BCG and values less than that reported by the manufacturer were found.

A micro-method used by one manufacturer in which .025 ml aliquots of suspensions of BCG are dropped on growth medium has proved to be a very reproducible, rapid, economical and efficient procedure for counting number of viable organisms.

The other procedures, although as reproducible and as sensitive as the microdrop procedure are more time consuming and require more media.

Significance to Bio-Medical Research and the Program of the Division: These studies have determined a suitable procedure for checking manufacturer's claims of viability and have direct application to the control of potency of

the product.

Proposed Course of Project: (1) Check lots of BCG vaccine submitted by manufacturer. (2) Characterize strains of mycobacteria with vaccine potentiality with regard to stability, immunogenicity and other characteristics important in the selection of a reference or standard preparation.

Honors and Awards: None

Publications: None

Serial No. DBS 102
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Immunology of the delayed contact sensitivity induced by catechols including those related to and contained in poison ivy extracts

Previous Serial Numbers: DBS 8, 9

Principal Investigators: Harold Baer, Ph. D.
Roy C. Watkins, M. S.

Other Investigators: None

Cooperating Units: Laboratory of Nutrition and Endocrinology (NIAMD)
Radiation Safety Department (CC)

Man Years

Total:	2.4
Professional:	1.7
Other:	0.7

Project Description:

Objectives: To examine the composition and to establish a method for the standardization of poison ivy extracts. To determine whether a poison ivy extract can hyposensitize humans. To determine the relationship of structure of catechols to their effectiveness in inducing delayed contact sensitivity and to study the mechanism of delayed contact sensitivity.

Methods Employed: (1) Guinea pigs, sensitized by injection with synthetic pentadecylcatechol (PDC) in Freund's adjuvant, were used to compare the reactivity of an extract with the reactivity of dilutions of PDC. (2) Large quantities of poison ivy leaves were purchased, extracted, and the extract sent to Prof. Charles Dawson at Columbia University who, under a contract with DBS, studied its composition. Testing of components isolated by Prof. Dawson was carried out at DBS.

A commercially obtained poison ivy extract, which was standardized by the guinea pig potency test was diluted and, under contract with Dr. Robert Auerbach of the Orentreich Medical Group in New York City, given to sensitive humans both by injection and orally in capsules.

Synthetic catechols of varying structure (based on the structure of the active components of poison ivy extract) were prepared, under contract, by Prof. Charles Dawson and injected into guinea pigs to attempt sensitization. Each pig was tested not only for reactivity to the homologous catechol but also to the other catechols.

Urushiol, obtained from poison ivy leaves by Prof. Dawson was catalytically reduced in the presence of tritium to yield radioactive PDC and placed on the skin of normal guinea pigs. At various times the animals were sacrificed and the radioactivity of the organs studied.

Major Findings: (1) A potency test for poison ivy extracts was devised and sent to interested manufacturers for evaluation. (2) Prof. Dawson found that extracts of fresh poison ivy leaves contain urushiol as anticipated. Extracts of old leaves as well as a commercial extract contained no urushiol although assay revealed activity in these materials as measured by the skin-reactivity in sensitive guinea pigs. These extracts contain what appears to be degradation products of urushiol. Whether these arise subsequent to picking the leaves or are present in leaves late in the season remains to be determined.

By guinea pig potency assay, the extract did not change in potency over a one year storage period. Although treatment of some humans has covered a period of more than a year, there is no evidence of marked reduction of skin reactivity.

Most of the synthetic catechols tested could induce sensitivity in guinea pigs. Cross-reactivity, however, was limited to substances of very similar chemical structure.

Use of tritiated PDC revealed that PDC is rapidly disseminated from a local skin site.

Significance to Bio-Medical Research and the Program of the Division: The poison ivy potency test, if adopted, will be the first potency test for an Allergenic Product.

Chemical studies should eventually reveal how to obtain undegraded active components of ivy leaves. Studies with synthetic and tritiated PDC will reveal the mechanisms of sensitization with these compounds and which will be suitable for desensitization.

Proposed Course of Project: The project will be continued as outlined above.

Honors and Awards: None

Publications:

Baer, H., Watkins, R. C., and Bowser, R. T.: Delayed contact sensitivity to catechols and resorcinols. The relationship of structure and immunization procedure to sensitizing capacity. Immunochemistry. (In press)

Serial No. DBS 103
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Diagnostic skin test reagents other than tuberculin

Previous Serial Numbers: DBS 10, 11

Principal Investigators: Harold Baer, Ph. D.
Sotiros D. Chaparas, Ph. D.
Robert W. Kolb, M. S. (LCA)
Vernon J. Fuller, Ph. D. (LCA)

Other Investigators: Jack C. Jenkins (LCA)
Roy C. Watkins, M. S.

Cooperating Unit: Laboratory of Control Activities

Man Years

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: To develop suitable laboratory procedures and reference preparations for use in the potency testing of coccidioidin and toxoplasmin.

Methods Employed: During the current year a second lot of freeze-dried coccidioidin was prepared from unheated culture filtrates provided by Dr. Chester W. Emmons (NIAID). Guinea pigs were sensitized by injection, into the nuchal area, of coccidioidin in varying concentrations incorporated into Freund's adjuvant. The animals were then skin tested with a commercial preparation and the two experimental lots of coccidioidin.

Guinea pigs were sensitized to toxoplasmin by injecting toxoplasmin emulsified in complete Freund's adjuvant.

Major Findings:

Subproject 1: Preparation and standardization of coccidioidin (Harold Baer, Robert W. Kolb, and Vernon J. Fuller). Skin reactions were elicited by a licensed commercial preparation in guinea pigs sensitized with an experimental lot of coccidioidin.

Titration of comparable concentrations of the two experimental lots

of coccidioidin showed differences in potency. These differences may be due to (1) degree of purity of the materials, (2) stability differences, or (3) possible suppressive interaction of the test materials. These data are being evaluated and will be reported. The second lot revealed more gel diffusion bands than the first indicating that it was antigenically different from the first lot.

Subproject 2: Preparation and standardization of toxoplasmin (Sotiros D. Chaparas, Robert W. Kolb, and Vernon J. Fuller). (1) Delayed skin reactivity to toxoplasmin was induced in guinea pigs. Toxoplasmins from cell sources other than that used for skin testing were effective in sensitizing the animals. (2) The procedure for sensitization although very effective in sensitizing virtually all animals is limited in that commercial preparations do not induce significantly large reactions in all animals. The reason for low reactivity is that commercial preparations for human use contain but very small quantities of toxoplasmin detectable in only the more sensitive guinea pigs. (3) Weaker reacting skin test reagents are often suppressed when tested in animals along with more strongly reacting reagents. Toxoplasmins of approximately the same potency are often mutually suppressing.

Significance to Bio-Medical Research and the Program of the Division: It is anticipated that the new test procedure will have application in the regulation, control, and standardization of commercial coccidioidin and toxoplasmin preparations and may prove applicable to other skin test reagents such as histoplasmin, trichinellin, etc.

Proposed Course of Project: Further studies to resolve the differences in the responses to various coccidioidins noted above are indicated. After these differences are resolved it is proposed that an adequate supply of coccidioidin be acquired for use as a control reference and for use in further studies.

To prepare toxoplasmins in primary monkey kidney cultured cells and primary chick embryo fibroblasts using chick embryo adapted toxoplasma. To investigate methods of increasing the sensitivity so that concentrations used for human testing may be tested directly with use of guinea pigs. To standardize toxoplasmin activity with regard to number of viable and non-viable organisms present in suspension prior to extraction and to work toward development of a reference preparation.

Honors and Awards: None

Publications:

Chaparas, S. D., Fuller, V. J., and Kolb, R. W.: A laboratory procedure for determining the potency of toxoplasmins for skin testing. Proc. Soc. Exp. Biol. Med. 121: 734-739, 1966.

Serial No. DBS 12
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Investigation of tetanus toxoids for prevention of neonatal tetanus

Previous Serial Number: DBS 12

Principal Investigators: Margaret Pittman, Ph. D.
Michael F. Barile, Ph. D.
M. Carolyn Hardegree, M. D.

Other Investigators: Frank Schofield, M. D., New Guinea
Robert MacLennan, M. D., New Guinea

Cooperating Units: Department of Public Health, Territory of Papua and New Guinea

Man Years

Total:	0.8
Professional:	0.3
Other:	0.5

Project Description:

Objectives: Neonatal tetanus is an important cause of infant mortality in New Guinea. Prevention by means of vaccination of pregnant women was found to be effective and the findings have been published. The study was extended to determine the persistence of the antitoxin titers of the first-study women, and of women of childbearing age vaccinated with oil-emulsified and adsorbed toxoids, and the booster response of the women to adsorbed or plain toxoid injected during pregnancy.

Methods Employed: The mouse neutralization and hemagglutination tests were used to determine antitoxin levels of the sera of the women.

Major Findings: (1) With the use of lots of toxoids different from those in the first phase of the study, antitoxin levels were higher at 8 and 18 months in those women who received one injection of the oil-adjuvant toxoids than in those who received two injections of the adsorbed toxoid. The latter, however, were above the protective level against neonatal tetanus. (2) A high incidence of abscesses occurred (75%) in recipients of the second

lots of oil-emulsified toxoids. (3) An anamnestic response (10-fold rise or greater) was obtained with booster injections of either adsorbed or plain toxoid; the response to the adsorbed toxoid was greater than to the plain toxoid.

Significance to Bio-Medical Research and the Program of the Division: Neonatal tetanus can be prevented by the immunization of pregnant women. Two injections of adsorbed toxoids containing approximately 200 units induces antitoxin titers similar, or not different from a protective standpoint, to those induced by one injection of oil-emulsified tetanus toxoids and the protective levels persist for at least two years while titers induced by three injections of plain toxoid may fall below the estimated protective level of 0.01 unit/ml in less than one year. Until it is known how to detect and inhibit the abscess-inducing property of lots of oil-emulsified toxoid, aluminum-salt adsorbed toxoid is safer and when administered in two doses is equally as protective for at least two years as one dose of oil-adjuvant toxoid.

The field and laboratory results provide information on the relation of human antitoxin response to the laboratory assayed potency of tetanus toxoids. The findings are applicable to the potency and safety specifications for tetanus toxoid.

Proposed Course of Project: (1) To publish the laboratory and clinical findings, (2) to continue studies to determine duration of the protective antitoxin level and the booster response to adsorbed and plain toxoids, and (3) to determine cause of untoward reactivity of oil-adjuvants (Proj. No. 15).

Honors and Awards: None

Publications:

MacLennan, R., Schofield, F., Pittman, M., Hardegree, M. C., and Barile, M. F.: Immunization against neonatal tetanus in New Guinea: antitoxin response of pregnant women to adjuvant and plain toxoids. Bull. Wld Hlth Org. 32: 683-697, 1965. (Reported as "in press" in 1965.)

Serial No. DBS 13
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A study of the antigenic substances of Clostridium tetani

Previous Serial Number: DBS 13

Principal Investigator: M. Carolyn Hardegree, M. D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description:

Objectives: (1) To determine and separate the antigens present in culture filtrates of Clostridium tetani, in particular neurotoxin from hemolysin. (2) To characterize the in vivo and in vitro activities of these substances. (3) To determine their relationship to optimal anti-tetanus immunization.

Methods Employed: Column chromatography, zone electrophoresis, gel diffusion, immunoelectrophoresis, enzyme assays, plasma hemoglobin determination, animal bioassay, animal immunization.

Major Findings: (1) Neurotoxin and hemolysin was separated from culture filtrates of the Massachusetts C_2 strain by use of Sephadex gel filtration. The ratio of hemolysin to neurotoxin was increased at least a thousand fold. (2) The fractions with maximum hemolytic activity caused death within 10-15 minutes when injected intravenously into mice, and hemolysis in vivo when injected into rabbits. In vivo studies in monkeys were recently initiated (Dr. A. E. Palmer, LP). Toxoids of culture filtrates induced the formation of an anti-hemolysin in rabbits. Some commercial equine antitoxins and human tetanus immune globulin contained antihemolytic substances. (3) Esterase activity was detected in crude culture filtrates by use of olive oil and Arlacel A, mannide monoleate, as substrates. This enzyme is distinct from the neurotoxin and hemolysin. (4) A gelatinase was separated by gel filtration from the neurotoxin, hemolysin and esterase.

Significance to Bio-Medical Research and the Program of the Division: It is expected that the investigation will contribute to an understanding of the antibodies against Cl. tetani antigens other than neurotoxins. These antibodies may be important in the antitoxin treatment of tetanus as well as in tetanus prophylaxis. Findings will provide fundamental information for the control of these products.

Proposed Course of Project: Studies will be continued on (1) the isolation and recovery of components of the culture filtrates, (2) the continued characterization of the in vivo and in vitro effects of the hemolysin, and (3) the relation of the esterase to adverse reactions of antigens in oil adjuvants.

Honors and Awards: None

Publications:

Hardegree, M. C.: Separation of neurotoxin and hemolysin of Clostridium tetani. Proc. Soc. Exp. Biol. Med. 119: 405-408, 1965.

Serial No. DBS 14
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Standardization of reference preparations for tetanus toxoids

Previous Serial Number: DBS 14

Principal Investigators: Margaret Pittman, Ph. D.
Michael F. Barile, Ph. D.
M. Carolyn Hardegree, M. D.
Robert W. Kolb, M. S. (LCA)
Edward B. Seligmann, Jr., Ph. D. (LCA)

Other Investigators: None

Cooperating Units: Laboratory of Control Activities, DBS
World Health Organization

Man Years

Total:	0.6
Professional:	0.2
Other:	0.4

Project Description:

Objectives: By invitation, the Division of Biologics Standards collaborated in the standardization of the proposed international reference for tetanus toxoid adsorbed. The DBS objectives were not only to assist in determining the suitability of the international preparation but (1) to determine the potency of a U.S. reference tetanus toxoid in terms of the international preparations, (2) to establish a unitage for a U.S. reference toxoid, (3) to correlate laboratory assayed potency with the antitoxin response of women in New Guinea and (4) to relate these findings to the U.S. potency requirement for tetanus toxoid.

Methods Employed: Mice and guinea pigs were vaccinated with graded doses of tetanus toxoids and their responses were determined (1) by challenge with tetanus toxin and (2) by titration of antitoxin levels of guinea pig sera by the mouse neutralization test.

Major Findings: (1) Based on the results of the collaborators (including present investigators) WHO assigned a unit of potency to the toxoid

under consideration and designated it as the International Reference for Tetanus Toxoid Adsorbed. (2) Relative potency of adsorbed toxoids assayed with use of mice and guinea pigs were similar but the ED50s were two times greater with mice than with guinea pigs. (3) With the field trial toxoids, the unitages of the $AlPO_4$ adsorbed toxoids were six times greater than the unitage of the plain toxoids, and the antitoxin levels of the women, although similar at three months, were six times greater in those women vaccinated with adsorbed toxoid than in those vaccinated with plain toxoid at the end of 18-24 months.

Significance to Bio-Medical Research and the Program of the Division: The international reference tetanus toxoid adsorbed provides a basis for comparison of assayed potency of adsorbed tetanus toxoids prepared in all nations. (An international reference for the plain toxoids was designated earlier.) It will be possible to assay the unitage of U.S. tetanus toxoid by use of the correlated U.S. references. The finding that duration of antitoxin levels in man is directly related to the laboratory assayed unitage of the immunizing toxoid, if confirmed, will be helpful in establishing potency standards relative to human efficacy.

Proposed Course of Project: The findings will be prepared for publication. Attempts will be made to determine the feasibility of applying the information to the U.S. requirements for potency of tetanus toxoids.

Honors and Awards: None

Publications:

Pittman, M., Barile, M. F., and Hardegree, M. C.: Comparison of human response with laboratory assays of tetanus toxoid with and without adjuvants. Proceedings of Round Table Conference on Adjuvants, Utrecht, the Netherlands, 1966. (In press)

Serial No. DBS 15
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Investigation of toxicity of oil-emulsified antigens

Previous Serial Number: DBS 15

Principal Investigator: M. Carolyn Hardegree, M. D.

Other Investigator: None

Cooperating Units: None

Man Years

Total:	1.1
Professional:	0.6
Other:	0.5

Project Description:

Objectives: To determine factors responsible for the sterile abscesses seen in New Guinea women immunized with oil-emulsified tetanus toxoids. To examine the toxoids, as well as other vaccines containing an oil adjuvant, for free fatty acid (FFA) content. To examine biologics for their ability to hydrolyze Arlacel A, mannide monoleate.

Methods Employed: The methods of Duncombe and of Coleman-Middlebrook were used to assay free fatty acid in a variety of emulsified antigens and reaction mixtures of Arlacel A incubated with various biologics, either commercially or experimentally prepared.

Major Findings: (1) Toxoids and vaccines used in field studies where a high incidence of sterile abscesses was observed contained more free fatty acid (FFA) than did non-reacting emulsified antigens. (2) Free fatty acid was released from an emulsion of Arlacel A when it was incubated in the presence of certain antigens, e.g. tetanus toxin, cholera vaccine, and some allergenic extracts.

Significance to Bio-Medical Research and the Program of the Division: These studies provide information on the activity of components of oil-emulsified antigens and may be useful in developing specifications for oil-emulsified products should the use of oil adjuvants be approved.

Proposed Course of Project: To prepare findings for publication and to determine the relationship of the presence of free fatty acids in water-in-oil emulsified antigens to abscess formation in recipients.

Honors and Awards: None

Publications: None

Serial No. DBS 16
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The possible role of transitional L-forms of bacteria in human disease

Previous Serial Number: DBS 16

Principal Investigators: Michael F. Barile, Ph. D.
Edward A. Graykowski, D. D. S., M. D. (NIDR)

Other Investigators: Harold Stanley, D. D. S. (NIDR)
Winslow B. Lee, D. D. S. (NIDR)
Donald B. Riggs
Marion W. Grabowski
Constance DeYong, M. S. (NIDR)

Cooperating Unit: National Institute of Dental Research

Man Years

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To examine the role of transitional L-forms of streptococci in recurrent aphthae.

Methods Employed: (1) Direct cultural procedures were used to establish the prevalence of the transitional streptococci in lesions of aphthae and non-aphthae patients and in the saliva of normal subjects. (2) Twenty biochemical and four serologic methods were used to characterize the transitional streptococcus cultures. (3) Living and killed organisms and cell wall substance of the organisms were inoculated into laboratory animals for production of specific antisera.

Major Findings: (1) A transitional L-form of streptococcus was isolated from the lesions of 21/21 aphthae patients and from the lesions of 1/6 non-aphthae patients. Occasionally, this organism was recovered also from the saliva of patients with no history of recurrent aphthae. (2) The properties of 41 strains of the transitional streptococci tested indicated that the

stable-parent-organism of each strain was S. sanguis. This organism produced positive precipitin reactions with reference Streptococcus Group H antisera. (3) Living and killed S. sanguis and the Group C polysaccharide substance of this streptococcus induced anaphylactic and Arthus-type-skin hypersensitivity in guinea pigs and rabbits. A skin test vaccine prepared from heat-killed S. sanguis produced positive skin reactions in 31/31 aphthae patients tested but did not produce reactions in 6 non-aphthae patients.

Significance to Bio-Medical Research and the Program of the Division: These studies will be useful in establishing the etiology of recurrent aphthae and, subsequently, may provide a means by which this disease may be arrested, perhaps by vaccination. These studies are related to prevention and detection of Mycoplasma in biologics and provide suitable materials to test new and improved methods for primary isolation and detection of Mycoplasma and L-forms of bacteria.

Proposed Course of Project: To pursue studies to improve methods for isolation and detection of L-forms of bacteria in biologics.

Honors and Awards: None

Publications:

Graykowski, E. A., Barile, M. F., Lee, W. B., and Stanley, H. R.: The clinical, therapeutic, histopathologic and hypersensitivity aspects of recurrent aphthous stomatitis. J. Amer. Med. Assoc. (In press)

Serial No. DBS 17
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Mycoplasma in human disease and biologics

Previous Serial Number: DBS 17

Principal Investigators: Michael F. Barile, Ph. D.
Robert T. Schimke, M. D. (NIAMD)
Douglas R. Anderson, M. D. (NCI)
Gerald P. Bodey, M. D. (NCI)
Joseph Snyder, M. D. (NCI)
Hope H. Hopps, M. S. (DBS)
Barbara C. Bernheim, M. A. (DBS)

Other Investigators: Donald B. Riggs
Marion W. Grabowski

Cooperating Units: Laboratory of Virology and Rickettsiology, DBS
National Institute of Arthritic and Metabolic Diseases
National Cancer Institute

Man Years

Total:	1.6
Professional:	0.5
Other:	1.1

Project Description:

Objectives: (1) To examine the biology, metabolism, and pathogenicity of Mycoplasma. (2) To develop new methods or improve existing methods for primary isolation and detection of Mycoplasma as they pertain to the test for Mycoplasma required for certain biologic products. (3) To investigate the role of Mycoplasma in diseases of man and animals.

Methods Employed: (1) The presence of arginine dihydrolase activity in cultures of 61 Mycoplasma strains representing 18 serologically distinct Mycoplasma species was determined by use of chemical procedures. (2) The ultrastructures of selected mycoplasmata, rickettsiae, and ornithosis virus were examined by electron microscopy. (3) Bone marrow and blood from leukemic and non-leukemic patients were examined for presence of mycoplasmata by direct culture isolation procedures.

Major Findings: (1) Ten of 18 Mycoplasma species tested contained the arginine dihydrolase pathway. Different strains of the same species gave the same results. (2) On electron microscopic examination, Mycoplasma appeared pleomorphic, lacked a cell wall, possessed a 3-layer-unit membrane (as do animal cells), and had morphologic structures, such as, bud-formations, filamentous blebs, vacuoles containing smaller "reproductive bodies", which suggested several methods of reproduction. Rickettsiae and ornithosis virus had cell walls and were readily distinguishable from Mycoplasma. (3) Mycoplasma orale were isolated by direct cultural procedures from the blood and bone marrow of 4 of 10 leukemic patients; but they were not isolated from the blood and bone marrows of 10 non-leukemic patients.

Significance to Bio-Medical Research and the Program of the Division: (1) Studies on Mycoplasma morphology and isolation from bone marrow may provide information on whether or not Mycoplasma has an etiological role in human leukemia. (2) Presence of arginine dihydrolase activity may prove useful for purpose of classification and identification of Mycoplasma. (3) These studies are related to prevention, isolation, and detection of Mycoplasma in biologics which are subjected to Mycoplasma contamination risk.

Proposed Course of Project: To pursue studies to improve methods for primary isolation of Mycoplasma from biologics and tissues associated with disease. To consider assay methods for establishing requirements for Mycoplasma vaccine products.

Honors and Awards: None

Publications:

Anderson, D. R., and Barile, M. F.: Ultrastructure of Mycoplasma hominis. J. Bacteriol. 90: 180-192, 1965. (Reported as "in press" in 1965.)

Barile, M. F., Schimke, R. T., and Riggs, D. B.: Presence of the arginine dihydrolase pathway in Mycoplasma. J. Bacteriol. 91: 189-193, 1966.

Barile, M. F.: Mycoplasma (PPLO), Leukemia and Autoimmune Disease. In Defendi (ed) Methodological Approaches to Study of Leukemia. Wistar Inst. Press, Philadelphia, 1965, pp. 171-181.

Anderson, D. R., and Barile, M. F.: Ultrastructure of Mycoplasma orale isolated from patients with leukemia. J. Nat. Cancer Inst. 36: 161-167, 1966.

Barile, M. F., Bodey, G. P., Snyder, J., Riggs, D. B., and Grabowski, M. W.: Isolation of Mycoplasma orale from leukemic bone marrow and blood by direct culture. J. Nat. Cancer Inst. 36: 155-159, 1966.

Anderson, D. R., Hopps, H. E., Barile, M. F., and Bernheim, B. C.: Comparison of the ultrastructure of several rickettsiae, ornithosis virus and Mycoplasma in tissue culture. J. Bacteriol. 90: 1387-1404, 1965.

Serial No. DBS 104
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Standardization of pertussis vaccine

Previous Serial Numbers: DBS 18, 19, 21

Principal Investigators: Margaret Pittman, Ph.D.
Roberta A. Gardner, Ph.D.

Other Investigators: Claire B. Cox, M.S.
James F. Marshall
Ronald G. Clark, Ph.D., LP
Howard Bohner, LA-DRS

Cooperating Units: Laboratory of Pathology, DBS
Laboratory Aids Branch, DRS

Man Years

Total: 2.8
Professional: 1.2
Other: 1.6

Project Description:

Objectives: The objective is that all lots of products containing pertussis vaccine issued by U.S. licensed establishments have optimum properties: adequate and stable potency throughout the dating period and relative freedom from untoward reactive substances.

Methods Employed: Mouse potency tests to determine adequacy of potency and stability of potency. Analyses of results at intervals to determine any reflected trend. Measurement of pertussis vaccine toxicity by determination of weight changes in mice, determination of the lethal dose in mice, and the production of necrosis in the skin of rabbits and suckling mice. Serotyping of Bordetella pertussis strains.

Major Findings: 1. No significant changes in potency and toxicity of the pertussis vaccine components in vaccines submitted for release were noted. (Cox and Marshall.)

2. Two candidate toxicity references for pertussis vaccine have been procured and characterized. Plans are being made for a field trial to correlate human reactivity to pertussis vaccine with laboratory measured toxicity. Toxicity references used in Japan and England have been tested in

comparison with our two candidate toxicity references. (Gardner and Pittman.)

3. The mouse strain in which pertussis vaccine is tested has a significant effect upon the apparent toxicity of the vaccine. The bacterial strain used for production of pertussis vaccine has some influence on the toxicity of the vaccine. (Gardner and Pittman.)

4. Germfree mice were significantly less susceptible to the toxic effects of pertussis vaccine than were conventional mice of the same strain. (Gardner, Clark, Bohner.)

5. Experimental pertussis vaccines containing various concentrations of Merthiolate were prepared, in order to determine whether concentrations of Merthiolate other than those routinely used would have a greater effect in conserving pertussis vaccine potency. After one year of storage at 4° C there were no significant differences in the potency of vaccines preserved with different concentrations of Merthiolate. (Gardner and Pittman.)

6. Serotyping of strains of B. pertussis in current use for vaccine manufacture in the U.S. and of strains isolated from patients is being carried out under a contract with Dr. Grace Eldering of the Michigan State Department of Health. Results of this study are not yet available.

Significance to Bio-Medical Research and the Program of the Division: The surveillance of and research on pertussis vaccine provides information relating to control and improvement of the product and basic scientific information about immunological and reactive properties of B. pertussis.

Proposed Course of Project: Emphasis will be placed on investigating the relation of laboratory measured toxicity of pertussis vaccine to its reactivity in children. Adoption of a toxicity reference for pertussis vaccine is expected. Serotyping of B. pertussis (under contract) will continue.

Exchange of information and cooperative testing with international laboratories, particularly in reference to the relation to laboratory assayed toxicity and human reactivity of pertussis vaccine, will be continued. A project on correlation of laboratory-assayed toxicity with human reactivity to be carried out jointly with the Division of Biologics Standards and the Division on Human Bacterial Products, Pharmaceutical Manufacturers Association is in the formative stage.

Honors and Awards: None

Publications:

Gardner, Roberta A., and Pittman, Margaret: Relative Stability of Pertussis Vaccine Preserved with Merthiolate, Benzethonium Chloride, or the Parabens. Appl. Microbiol. 13: 564-569, 1965.

Serial No. DBS 22
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Immunologic protection of the infant rabbit against experimental cholera

Previous Serial Number: DBS 22

Principal Investigator: John C. Feeley, Ph.D.

Other Investigator: Charles O. Roberts

Cooperating Unit: World Health Organization

Man Years

Total: 0.5
Professional: 0.4
Other: 0.1

Project Description:

Objectives: To investigate the role of antibody in passive protection of the orally-infected infant rabbit and to examine the potential of this animal model for the assay of cholera vaccines.

Methods Employed: Antiserum pools from adult rabbits repeatedly immunized with three field trial cholera vaccines were assayed on a qualitative basis for passive protective activity in the orally-infected infant rabbit as described previously. Sera were also titrated for agglutinating (7S and 19S), vibriocidal, and skin toxin (permeability factor) neutralizing antibodies using published methods.

Major Findings: For each of the 3 field trial vaccines, serum pools obtained from adult rabbits "early" (14 days) and "late" (56 days) in the course of immunization were tested, since "late" sera from animals immunized with living vibrios were found previously to be more protective against death. In the present experiments, neither "early" or "late" serum pools prepared against any of the 3 field trial vaccines showed significant protection against death or significantly extended survival time as compared to the control group. One of these vaccines (CRL) showed good protection in man in the CRL (1963-64) field trial, yet it did not induce antibody giving a high degree of infant-rabbit passive protection. The other 2 vaccines,

employed in the Calcutta field trial (1965), gave inconclusive results in the field for reasons unknown. Hence, the method employed failed to show that passive protection in the infant rabbit model could be related to the ability of a vaccine to protect man.

The lack of passive protective activity of these 3 "anti-vaccine" sera was in sharp contrast to the high degree of protection previously shown with sera prepared by immunizing rabbits with living V. cholerae. In that instance, a high degree of passive protective activity was manifest by "late" but not "early" sera.

The agglutinating and vibriocidal antibody titers of the "early" and "late" serum pools from rabbits immunized with cholera vaccines were comparable to those of the "early" and "late" serum pools from animals immunized with living vibrios, hence neither vibriocidal nor agglutinating antibody levels correlated with passive protective activity for the infant rabbit. A comparable change of 19S to 7S antibodies also occurred between "early" and "late" pools from animals receiving either vaccines or live vibrios. Hence, the earlier hypothesis that the increase in protective activity between "early" and "late" pools might be due to a shift from 19S to 7S antibodies seems unlikely. It was observed that "late" sera from rabbits immunized over a prolonged period with living vibrios did have skin toxin neutralizing power, while sera from rabbits similarly immunized with the 3 vaccines showed no antitoxin activity, hence a rise in antitoxin titer appeared to correlate with passive protective power. However, it would be hazardous to conclude that "antitoxin" alone was responsible for protective activity, since "late" protective sera also show many more bands in agar gel diffusion tests than "early" nonprotective sera; this implies that increases in a large number of other antibodies to a variety of V. cholerae antigens have also occurred.

Significance to Bio-Medical Research and the Program of the Division: It is apparent that even prolonged immunization of rabbits with a vaccine of proven efficacy in man fails to yield a serum of significant passive protective activity for infant rabbits when tested by the method described. Hence, the value of this method for assay of vaccine is open to question. Furthermore, the method is too cumbersome, time-consuming, and expensive for routine application. However, because of the unique similarity of the disease in the infant rabbit and in man, the model may still be of value for immunologic studies on the mechanism of antibody-mediated protection within the bowel.

Proposed Course of Project: Studies are contemplated to determine the role of different antibodies (e.g. agglutinating or vibriocidal antibodies and antitoxin) in protection and to shed some light on the nature of the immune mechanism operating within the bowel.

Honors and Awards: None

Publications:

Feeley, J. C.: Passive protective activity of antisera in infant rabbits infected orally with Vibrio cholerae. In Proceedings of the Cholera Research Symposium, Public Health Service Publication No. 1328, U. S. Government Printing Office, Washington, D. C., 1965, pp. 231-235.

Serial No. DBS 23
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Standardization of cholera vaccine

Previous Serial Number: DBS 23+ (24 & 25)

Principal Investigator: John C. Feeley, Ph.D.

Other Investigators: Margaret Pittman, Ph.D.
Charles O. Roberts

Cooperating Units: NIH Cholera Advisory Committee
Pakistan-SEATO Cholera Research Laboratory
World Health Organization
Laboratory of Biophysics and Biochemistry, DBS
Laboratory of Control Activities, DBS

Man Years

Total: 1.0
Professional: 0.7
Other: 0.3

Project Description:

Objectives: Establishment of an improved U. S. Standard of Potency and development of other requirements pertinent to manufacture and standardization of cholera vaccine.

Methods Employed: Quantitative potency tests and toxicity tests in mice. Chemical assays of nitrogen content on dialyzed and undialyzed vaccines and on material precipitated from vaccines by 1.5 volumes of acetone.

Major Findings: (1) Mouse protection assays revealed that the fluid El Tor vaccine was slightly more potent than the fluid classic cholera vaccine employed in the 1964 Philippine field trial; however, the difference was not statistically significant. Both vaccines afforded equal protection shortly after injection against Ogawa El Tor strain infection (63%) in the field trial, but the El Tor vaccine showed somewhat more lasting protection. Protection by either vaccine was nil at 6 months. Both vaccines were only slightly less potent on a human dose basis against Ogawa challenge than the vaccine employed in the CRL field trial. The CRL vaccine gave 72% protection

against infection caused by classical Inaba strains of V. cholerae and protection was evident at least 18 months after immunization. It is not known whether the greater and more long-lasting protection given by the CRL vaccine in the field was due to differences in vaccines, to response of the different population groups, or to basic differences in ability of vaccines to protect against Inaba and Ogawa serotypes.

(2) Mouse protection assays were completed on the four vaccines employed in the 1965 Calcutta field trials. One of these vaccines was significantly more potent than the other 3 which differed only slightly from each other in potency. The most potent vaccine was comparable in potency, on a human dose basis, to the CRL field trial vaccine, it had a higher bacterial content, was also the most toxic in mice. The field trial was conducted in two phases; in the first phase, two of the three lower potency vaccines were compared. One afforded better protection than the other in the field, but neither showed a significant difference compared to the typhoid vaccine placebo. The vaccine which gave apparently better protection in man was slightly, but not significantly, higher in mouse potency. The second phase of the field trial, which compared the vaccine of highest potency with the third vaccine of lower potency, was a failure for reasons unknown. Neither vaccine showed any evidence of protection compared with the typhoid placebo group, but the number of cases of cholera was small.

(3) Data collected thus far in comparative assays in this laboratory and in LCA on vaccines submitted for release by U. S. licensed manufacturers show a general agreement in results between laboratories, but a more extensive collection of results obtained over a longer period of time will be necessary to evaluate fully the degree of agreement between laboratories and between DBS and manufacturers' tests.

(4) Results of nitrogen assays on cholera vaccines show fairly close agreement of values for non-dialyzable nitrogen and acetone-precipitable nitrogen on most cholera vaccines. Both methods would appear to have value for controlling the bacterial content of vaccines, but the acetone precipitation procedure is technically advantageous. However, the latter method was not satisfactory for nitrogen assay of one U. S. licensed vaccine due to non-dialyzable and acetone-precipitable nitrogen in the broth in which the organisms are grown and which is present in the vaccine.

Significance to Bio-Medical Research and the Program of the Division: The present project is part of an effort to correlate the results of laboratory assays of cholera vaccine with efficacy in man. Eventual achievement of this goal is necessary to exert meaningful control over the potency of cholera vaccine, and to formulate adequate Additional Standards for inclusion in Regulations.

Proposed Course of Project: To carry through as indicated above. Additional studies of vaccines employed in field trials which yield statistically significant results will be required to establish the

relationship of laboratory potency and prophylactic efficacy. Since field trial results, excluding those from East Pakistan, indicate a failure of cholera vaccines to provide a high degree of protection of adequate duration, it is anticipated that new types of vaccines containing aluminum adjuvants or toxoids (of choleragenic "toxin") will be explored.

Honors and Awards: None

Publications:

Pittman, M., and Feeley, J. C.: Laboratory assays of cholera vaccine potency. In Proceedings of the Cholera Research Symposium, Public Health Service Publication No. 1328, U. S. Government Printing Office, Washington, D. C., 1965, pp. 163-167. (Reported as "in press" in 1965.)

Feeley, J. C.: Comparison of vibriocidal and agglutinating antibody responses in cholera patients. In Proceedings of the Cholera Research Symposium. Public Health Service Publication No. 1328, U. S. Government Printing Office, Washington, D. C., 1965, pp. 220-222. (Reported as "in press" in 1965.) Work was completed in 1962. Previous Serial No. 24.

McIntyre, O. R., Feeley, J. C., Greenough, W. R., III, Benenson, A. S., Hassan, S. I., and Saad, A.: Diarrhea caused by non-cholera vibrios. Am. J. Trop. Med. & Hyg. 14: 412-418, 1965. (Reported as "in press" in 1965.) Work terminated in 1963. Manuscript prepared in 1964-1965. Previous Serial No. 25.

McIntyre, O. R., and Feeley, J. C.: Characteristics of non-cholera vibrios isolated from cases of human diarrhea. Bull. Wld Hlth Org. 32: 627-632, 1965. (Reported as "in press" in 1965.) Work terminated in 1963. Manuscript prepared in 1964-1965. Previous Serial No. 25.

Serial No. DBS 26
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Characterization of strains of Vibrio cholerae

Previous Serial Number: DBS 26

Principal Investigator: John C. Feeley, Ph.D.

Other Investigators: Charles O. Roberts

Cooperating Units: None

Man Years

Total: 0.1
Professional: 0.1
Other: 0

Project Description:

Objectives: To study the subspecies characteristics of strains of Vibrio cholerae from recent outbreaks of cholera.

Methods Employed: Routine bacteriological and serological procedures, augmented by tests for bacteriophage sensitivity and hemagglutinating and hemolytic activities.

Major Findings: During this year 68 strains of V. cholerae isolated during the 1965 cholera outbreak in Iran were studied. All strains belonged to type 4 (phage IV resistant, VP Positive, chicken erythrocytes agglutinated; plate hemolysis positive, tube hemolysis negative) of the previously described scheme, and would be regarded by some workers as "nonhemolytic" or "weakly hemolytic" El Tor vibrios. Sixty of the 68 cultures were single isolates from different patients and all were of the Ogawa serotype. The remaining 8 cultures represented an interesting series obtained from one patient over a 2 week period: 2 were Ogawa (serotype AB), 3 were Inaba (serotype AC), and 3 were Hikojima (serotype ABC), apparently representing mutants of the Ogawa type primarily responsible for the outbreak. These are the first authentic Hikojima strains encountered, despite examination of many labeled as such, in hundreds studied in this laboratory over the past several years. Six Ogawa type 4 strains isolated in Viet-Nam in 1965 were examined, also.

Significance to Bio-Medical Research and the Program of the Division: The present findings represent a further accumulation of information on the epidemiologic value of the proposed subspecies classification scheme. It is possible to show that type 4 strains, first recognized in Malaysia and Thailand in 1963, have moved successively into Viet Nam, Burma, India (1964-1965), West Pakistan (1964--not reported officially), and finally westward into Iran in 1965. In India, they may be replacing type 1 (classic *V. cholerae*) strains. Type 4 strains, which are only weakly hemolytic, differ from the strongly hemolytic (type 3) strains involved in the early phases of the Western Pacific epidemic wave beginning in 1961.

Proposed Course of Project: As they become available, representative strains from cholera outbreaks will be examined to determine their position in the proposed scheme.

Honors and Awards: None

Publications: None

Serial No. DBS 105
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on Vibrio cholerae toxin

Previous Serial Number: None

Principal Investigator: John C. Feeley, Ph.D.

Other Investigator: Charles O. Roberts

Cooperating Units: None

Man Years

Total: 0.7
Professional: 0.5
Other: 0.2

Project Description:

Objectives: To study the properties of toxic materials of possible pathogenic significance elaborated by Vibrio cholerae in relation to their possible value as immunizing antigens.

Methods Employed: Toxin was assayed by its ability to induce increased capillary permeability in guinea pig skin as determined by intravascular injection of Evans Blue dye. Toxin producing capacity of different strains and production of toxin under different cultural conditions were examined. Preliminary studies on inactivation of toxic activity and antigenicity of the material were carried out.

Major Findings: Strains of V. cholerae were found to vary significantly in toxin production. While all cultures tested elaborated some toxin, U. S. vaccine production strains (NIH 41 and 35A3) were poor producers, and an old Inaba strain (569B) widely used by others in study of cholera toxins was the best producer. Other strains of more recent isolation which had been maintained in the freeze-dried state with few transfers on media were intermediate in toxigenicity.

Confirming observations of others, best toxin production was obtained in shaken (aerated) broth cultures. Very little toxin was elaborated by

static broth or agar grown cultures. Best toxin production was obtained in the "Syncase" (synthetic salts medium plus 1% casamino acid) medium found optimum for production of "choleragen" (infant rabbit toxin) by Finkelstein. In aerated syncase cultures, toxin was detectable at 4 hours and reached a maximum at 18-24 hours, with no significant change in titer up to 72 hours. Only a trace amount of toxin was produced in the synthetic medium without casamino acids.

The toxin was almost completely inactivated by heat (60° - 30 min.), and by 0.4% formalin, but not by 0.5% phenol or by 1:10,000 Merthiolate. The toxic activity is very stable at 4° C for at least 3 months.

In preliminary antigenicity studies, it was found that rabbits and guinea pigs immunized with either toxic filtrates or experimental vaccines directly prepared from Syncase culture containing active toxin or toxoid (0.4% formalin inactivated) develop toxin neutralizing antibody. Guinea pigs also developed resistance to skin testing with toxin following immunization.

Rabbits and guinea pigs immunized with cholera vaccines of U. S. manufacturers (including vaccine used in the successful CRL field trial) failed to develop significant antitoxin levels, although serum from rabbits hyperimmunized with living vibrios did contain antitoxin (Project no. 22). Guinea pigs did not develop resistance to skin testing after immunization with these commercial vaccines. The lack of antitoxin response to U. S. vaccine may be due to the poor toxigenicity of the culture employed and/or unfavorable conditions of cultivation during production.

Significance to Bio-Medical Research and the Program of the Division: At this time, based on our studies and those of others, it appears that toxin prepared as described can produce not only skin reactions in guinea pigs, but can also produce cholera in the infant rabbit and in ligated adult rabbit intestinal loop models as well as in human volunteers. These observations, coupled with the fact that similar toxic material is present in rice water stools of cholera patients, are highly suggestive that it may play a role in pathogenesis of the disease in man. In this light, it would appear that the theoretical possibility exists of improving the efficacy of cholera vaccine by including the toxoid. However, it seems likely that the antitoxic response is not solely responsible for immunity conferred on man by cholera immunization, since the CRL vaccine of known efficacy did not produce a significant rise in antitoxin on immunization of animals.

Proposed Course of Project: Further studies will be conducted on methods of producing and concentrating toxin and toxoid and on preparation of vaccines containing toxoid. These experimental vaccines will be assayed for potency in mice and for their ability to induce antitoxic, agglutinating and vibriocidal antibodies in rabbits and guinea pigs. Antisera against the vaccines also will be tested for passive protective activity against cholera in infant rabbits.

Honors and Awards: None

Publications: None

Serial No. DBS 27
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Investigations concerning staphylococcus antigens

Previous Serial Number: DBS 27

Principal Investigators: Claire B. Cox, M.S.
John C. Feeley, Ph.D.

Other Investigator: James F. Marshall

Cooperating Units: None

Man Years

Total: 0.50
Professional: 0.45
Other: 0.05

Project Description:

Objectives: (1) To make a survey of the literature pertaining to staphylococcus products and their clinical use, and (2) to carry out preliminary investigations on methods for measuring their potency.

Methods Employed: (1) Investigations were continued to evaluate a live antigen agglutination test to detect type specific antibodies in the sera of immunized rabbits. Nine rabbits (3 per serotype) were immunized by subcutaneous route with commercial staphylococcal phage lysate preparations: Serotype I, Serotype III and a mixture of I and III. The immunization schedule used was the manufacturer's recommended schedule for humans over a one-month period. The animals were bled prior to, midway and upon completion of immunizations. (2) The agar layer plaque count was further compared to the tube dilution method for measuring potency of staphylococcal phage containing products.

Major Findings: (1) The method of Boyer et al. (Proc. Soc. Exp. Biol. Med. 104: 659, 1960) using two-fold dilutions of inactivated sera and 100 VE50's (Viability Endpoint) of live antigen proved to be a satisfactory method for measuring serum levels of Staphylococcal Serotypes I, III and a mixture of I and III. (2) The agar-layer plaque count was found to give more reproducible and accurate results in measuring phage content of commercial

products than the tube dilution method.

Significance to Bio-Medical Research and the Program of the Division: The methods are applicable as assay procedures for certain staphylococcal products when "Standards for Staphylococcal Products" are specified.

Proposed Course of Project: If there is renewed interest in developing standards for staphylococcal products, further work is anticipated.

Honors and Awards: None

Publications: None

DCRT
LBB
LBBP
DREB
LCA
LP
LVI
LVR
CONTRACTS
SUBJECT INDEX
INVESTIGATORS
PUBLICATIONS

Annual Report of the
Laboratory of Biophysics and Biochemistry
Division of Biologics Standards
July 1965 through June 1966

A. Research Activities

I. Microbial Biophysics: Considerably reduced in scale over past years because of limitations in laboratory space and personnel, this part of the program nevertheless continued to contribute some research findings of interest. Theoretical investigations of problems dealing with mechanisms and kinetics of viral inactivation (Proj. No. 28, p. No. 55) were facilitated by a contract arrangement with Electronic Associates, Inc., Rockville, Md., which provided analog computer rental and engineering services. Laboratory experiments to test validity of model systems devised on the computer were confined mainly to bacteriophages to make most efficient use of the laboratory facilities.

II. Analytical Chemistry: Research activities of the group supervised by Dr. Jerome J. Helprin have been confined primarily to development or refinement of analytical methods. Some of the information obtained in the course of these efforts has contributed to an improved understanding of the chemical behavior of biological products. Analytical services performed in support of other research activities in DBS constituted an appreciable part of the laboratory program. Operation of the analytical ultracentrifuge (Proj. No. 31, p. No. 62) was the most prominent supporting activity, having contributed substantially to four other research projects within the Division.

III. Virus Characterization: Responsibility for this important phase of the laboratory activities has been assigned to Dr. Kendall O. Smith, who joined the staff in September. Dr. Smith is a virologist with special training and experience in electron microscopy. Using a variety of laboratory methods in addition to microscopy, Dr. Smith and his supporting personnel have continued to study architecture of the adenovirus capsid while diverting a portion of their efforts to contribute to the characterization of adeno-associated virus (AAV) a small DNA virus symbiotically related to adenovirus (Proj. No. 86, p. No. 64). The studies undertaken by this group are especially relevant to the production of viral vaccines, in which the occasional presence of adventitious agents has constituted a serious control problem. The capability of detecting contaminating viruses by direct physical observation and promptly and definitively characterizing new viruses morphologically is regarded as an important asset to the DBS program.

B. Control Activities

During the calendar year 1965, chemical analyses were performed on 114 samples of biological products prepared by 26 manufacturers, of which 20 were domestic commercial establishments, 4 were state laboratories, and 2 were foreign manufacturers. Twenty-two different biological products were analyzed by one or more of the 19 analyses which were then within the repertoire of the group. The results obtained from these analyses have been added to our continuing collection of data on the chemical composition of biologicals.

Of the 114 samples, 74 had been submitted to DBS to be considered for release, 4 were obtained from DBS stocks of excess samples and were analyzed as controls or to supplement other data, 29 were in a miscellaneous group containing purchased samples, samples from investigations, and samples submitted for consideration before licensing or with Investigative New Drug applications, and another group of 7 samples was obtained from the annual inspection program. The samples analyzed fell into the following groups:

1. Bacterial vaccines: cholera, mycoplasma, pertussis, plague, typhoid, typhoid and paratyphoid.

2. Viral vaccines: influenza, measles (live and inactivated), smallpox.

3. Rickettsial vaccines: Rocky Mountain spotted fever, typhus.

4. Antisera: antirabies serum, antivenin, Botulinus antitoxin.

5. Tuberculin PPD

6. Allergenic extracts

The chemical analysis group also participated in the control program through the review of protocols of biologicals submitted to DBS for release. Protocols for a variety of products were reviewed for compliance with the Minimum Requirements, and for lot-to-lot consistency in chemical composition. Several errors in data were found, and several progressive changes were noted in the composition of products, which resulted in requests to the manufacturers to correct their data or processes.

C. Contract Activities

Through a personal service contract with Dr. Wolfgang von Oettingen we have obtained toxicological evaluation reports on β-propiolactone, phenylmercuric borate, and pyridine. These reports, which are critical reviews of the published literature, will serve as the beginning of a series of open-ended files on the toxicology of chemically defined constituents of biological products.

Serial No. DBS 28
1. Biophysics and Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies of the inactivation of viruses.

Previous Serial Number: DBS 28

Principal Investigator: C. W. Hiatt, Ph.D.

Other Investigators: John M. Easton, M.D.

Cooperating Units: None

Man Years

Total: 3.8
Professional: 1.3
Others: 2.5

Project Description:

Objectives: (1) To gain information about the fundamental nature of the various biological properties of viruses through studying the mechanisms of inactivation of these properties by physical and chemical agents.

(2) To develop an understanding of processes of inactivation that may be applied to the preparation of viral vaccines.

Methods Employed: Various animal and bacterial viruses are cultivated in cell cultures and the infective fluids from these subjected to treatment with graded doses of the inactivating agent. Samples taken at the different dosage levels are assayed for infectivity, and the kinetic relationship between dose and survival ratio is established. From the characteristics of the survival curve inferences are drawn about the properties of the virus and the mechanism of the inactivating process.

Major Findings: (1) Electrical analog simulation of photodynamic inactivation kinetics. A set of differential equations was devised to express the survival ratio of photosensitized T_2 coliphage as a function of time, light intensity, oxygen tension, and three parametric rate constants representing respectively the inactivation rate, O_2-diffusion rate and rate of utilization of O_2 within the phage particle. A small general purpose analog computer (EAI TR-20 or TR-48) was programmed to solve these equations simultaneously and to plot any selected variable as a function of time. It was thus possible to achieve an understanding of the system and to select critical conditions for laboratory experiments. One of the most interesting conditions arises when T_2 coliphage, photosensitized to a given degree and equilibrated with O_2, is instantly diluted 1:100 in an oxygen-free solvent and exposed at the same time to high-intensity light. Oxygen concentration in the particle decreases as it is used up by the photochemical reaction and as it diffuses into the surrounding medium. For significant photodynamic inactivation (90% or more) to occur, the velocity constant for diffusion must be less than 10% of the velocity constant for inactivation. Since the latter is adjustable by controlling the degree of photosensitization of the phage, the former can be determined by successive approximations. Actual experiments under these conditions are now in progress and are expected to yield quantitative data describing permeability of the phage membrane to oxygen at various temperatures.

(2) Determination of sub-lethal photodynamic effects on T_3 coliphage. Photodynamic inactivation curves for T_3 coliphage sensitized with toluidine blue and irradiated under a variety of experimental conditions when analyzed objectively were found to fit closely to theoretical curves for a 4-target model, in which each of 4 critical sites must be destroyed in order to kill the phage. By terminating the irradiation at a selected point, it was possible to obtain a phage suspension in which virtually every surviving particle had 3 hits and was eligible for inactivation upon receiving a single additional hit. Suspensions of this type were prepared reproducibly and were found to be stable for 24 hours or more at 4°C. We have found as yet no cellular host which can repair the 3-hit sublethal damage. Experiments are in progress to determine whether this sublethal damage affects the susceptibility of the phage to other inactivating processes, which would disclose whether or not the same target sites are involved.

(3) Theoretical effects of aggregation upon the shape of virus survival curves. For a virus preparation containing aggregates of 1, 2, 3 ... n particles with a corresponding frequency of occurrence of C_1, C_2, $C_3 \ldots C_n$, the survival equation is

$$\frac{N}{N_o} = 1 - \sum_{1}^{n} C_n(1-e^{-kt})^n$$

for a first-order inactivation process if it may be assumed that a) the aggregates remain fixed in size, b) each aggregate counts as a single infectious unit as long as it contains one or more active virions, and (c) the velocity constant (k) for inactivation of a virion is independent of the size of the aggregate in which it occurs. Theoretical curves were calculated from published data on distribution of aggregates of various sizes in vaccinia virus preparations. Semi-logarithmic plots have one or more inflection points but

their curvature is so slight that they are essentially straight lines. Slopes of these curves depend upon the degree of aggregation. Apparent velocity constants for inactivation of highly aggregated preparations are about 25% less than the values for monodisperse suspensions of single particles, but no significant induction period can be observed.

(4) Studies of thermolabile SV40. Simian virus 40 (SV40) which had become associated with a strain of adenovirus 4, and which was previously recognized because of its unusual thermolability, was found to persist as a contaminant after 12 consecutive passages of the adenovirus 4 in the presence of antiserum against SV40. This finding provided additional support to the conclusion that the SV40 genome is physically incorporated within the adenovirus capsid.

Significance to Bio-Medical Research and the Program of the Division: The continued investigation of the mode of action of viral inactivating processes is expected to contribute to the fundamental basis for control of viral vaccines.

Proposed Course of Project: This project will be continued.

Honors and Awards: None

Publications:

Hiatt, C. W., and Moore, D. E.: Virus-free infective centers produced by photodynamic inactivation of poliovirus in rhesus kidney cell suspensions. Proc. Soc. Exp. Biol. Med. 119: 203-205, 1965.

Easton, J. M., and Hiatt, C. W.: Possible incorporation of SV40 genome within capsid proteins of adenovirus 4. Proc. Nat. Acad. Sci. 54: 1100-1104, 1965.

Easton, J. M., and Hiatt, C. W.: Simian virus 40: replication in the presence of specific antiserum and adenovirus 4. Science 151: 582-583, 1966.

Serial No. DBS 85
1. Biophysics and Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Development of porous glass powders for steric exclusion chromatography.

Previous Serial Number: None

Principal Investigator: C. W. Hiatt, Ph.D., in collaboration with Wolfgang Haller, Ph.D. (NBS)

Other Investigators: Martin Harwin, M.D. (LVR)

Cooperating Units: 1. Glass Section, National Bureau of Standards, Wash., D.C.
2. Laboratory of Virology and Rickettsiology, DBS

Man Years

Total: 1.5
Professional: 0.5
Others: 1.0

Project Description:

Objectives: To explore the potential applications of glass powders of controlled pore size as matrices for steric exclusion chromatography of biological substances.

Methods Employed: Glass powders of various uniform pore sizes and with surfaces conditioned by various chemical treatments are used in column or batch experiments at controlled pH, ionic strength and specific ion concentration to attempt the separation and purification of bacteria, viruses, proteins, polysaccharides, and other macromolecular substances.

Major Findings: (1) Experiments with animal viruses. In chromatographic columns of glass with average pore diameters of 960 Å or 1800 Å, respectively, attempts were made to separate poliovirus or vesicular stomatitis virus by exclusion using various diluents. Both of these viruses adsorbed avidly to the glass and did not migrate through the column, even at high pH, high salt concentration, or in the presence of Tween 80. Very high concentrations (1-2 M) of choline, however, blocked the adsorption of poliovirus and would elute previously adsorbed virus. This led us to try various methods for chemical modification of the glass surface to introduce amino or methyl groups, for example. Methylation of the glass did not improve performance, but amination, when carried far enough, appeared to inhibit adsorption of poliovirus. Further experimentation will be necessary to devise methods for coupling amino groups to glass surfaces while still retaining the high degree of stability which is a notable virtue of the glass powders.

(2) Characterization of column performance. In order to predict chromatographic behavior as a function of molecular size, experiments were undertaken with a homopolymeric series of high-molecular-weight dextrans. No adsorption to the glass surfaces occurred. Elution positions for the various dextrans ranging in molecular weight from 10^4 to 10^6 were determined on porous glass columns of 5 graded pore diameters ranging from 125 Å to 1800 Å. The data obtained make it possible to estimate the molecular weight of an unknown substance on the basis of its elution position.

Significance to Bio-Medical Research and the Program of the Division: Glass-powder columns for exclusion chromatography, because of their uniformity and stability, large pore diameter, and rapid flow rates are potentially valuable for both the preparation and analysis of biological products.

Proposed Course of Project: This project will be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 29
1. Biophysics and Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Development of methods for the chemical analysis of biological products.

Previous Serial Number: DBS 29

Principal Investigator: Jerome J. Helprin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total: 3.00
Professional: 1.0
Others: 2.0

Project Description:

Objectives: To develop new methods of chemical analysis of biological products or to modify existing methods, thus facilitating the study of chemical problems in the control of and research on biologicals.

Methods Employed: Standard methods of chemical analysis are used, evaluated, or modified as required for the material being analyzed, and new methods are developed.

Major Findings:
(1) Studies have continued on an original method to measure mercurial preservatives in biologicals. The method employs the atomic absorption spectrophotometer to determine the amount of mercury present. The procedure is now being evaluated on various vaccines.

(2) An experimental Mycoplasma vaccine was analyzed for the toxic heavy metal thallium, of which there was a residuum from the culture medium. By use of the atomic absorption spectrophotometer an easily measurable amount of thallium was found, whereas the manufacturer had claimed it to be undetectable by his method of analysis.

(3) Our analytical method for phenol was modified to permit analyses of biologicals in which the protein content was so high that it interfered with the test. It was found that phenol could be extracted quantitatively by dialyzing it out of the sample and analyzing the dialysate. This has increased our capability for phenol analyses to include antisera and tuberculin PPD.

(4) Two methods of analysis for the preservative Benzethonium chloride were evaluated and the method having the greater reproducibility was adopted. In the course of this work it was found that most of the preservative was attached to readily sedimentable particulates in the vaccine rather than being present in the fluid menstruum, as one would have expected from the literature.

(5) An analytical method for formaldehyde was evaluated and added to our repertoire of methods for the control of biologicals.

(6) A method to measure protein nitrogen directly, rather than by difference between total and non-protein nitrogen was devised for use on the Technicon AutoAnalyzer. The protein was precipitated, removed from solution, redissolved, and analyzed for total nitrogen, in this case presumably being equal to protein nitrogen. The method is still under study.

(7) Direct protein determinations using the Folin-Ciocalteau reagent in the method developed by Lowry et al. have also been added to our repertoire.

(8) An electrolytic apparatus to measure moisture in dried biologicals was put into service to be evaluated and compared with conventional methods. This study is continuing.

Significance to Bio-Medical Research and the Program of the Division:
The significance of this work lies in providing increased knowledge of the chemical composition of biologicals which can be of value in research, and in surveillance of these products for the protection of the public.

Proposed Course of Project: This project will be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 31
1. Biophysics and Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Physical characterization of biological substances.

Previous Serial Number: DBS 31

Principal Investigator: Andrew M. Young, M.S.

Other Investigators: None

Cooperating Units: None

Man Years

Total: 1.0
Professional: 1.0
Others: 0

Project Description:

Objectives: To obtain information about the physical dimensions of biological substances and to correlate this information with biological properties.

Methods Employed: Standard methods of physical analysis, including ultracentrifugation, are supplemented by specialized techniques for fractionating and concentrating proteins and other macromolecular substances.

Major Findings:
(1) Sedimentation constants were determined to test the purity of a variety of prothrombin preparations and to characterize certain chromatographic fractions thereof. In collaboration with Dr. Aronson of LBBP 29 analyses were performed on the ultracentrifuge; sedimentation constants ranged from 4.34×10^{-13} to 5.59×10^{-13}.

(2) A group of 5 sedimentation velocity experiments were run in collaboration with Dr. Finlayson of LBBP on the perchloric acid supernatants of barium-treated human sera. The sedimentation constants ranged from 2.29×10^{-13} to 2.75×10^{-13} and were used as a means to characterize fractions, as a criterion of purity, and for identification purposes.

(3) A group of 37 sedimentation velocity experiments were performed in collaboration with Dr. Li of LVR on fractions of clam extract purified on sephadex. The sedimentation constants were calculated, the diffusion coefficients were approximated, and the molecular weights of the various fractions were estimated. The fractions were found to range from a molecular weight of 500 to 12,000.

(4) A group of 22 samples of γ-globulin from various manufacturers were examined by means of sedimentation velocity experiments. The samples were found to have components ranging from 3.27×10^{-13} to 10.64×10^{-13}. Some samples had 3 components, others only 2. The main difference, however, was the amount of the components rather than the type contained. This work was done in collaboration with Mr. Art of LBBP.

Significance to Bio-Medical Research and the Program of the Division: Physical characterization of biological products is part of the basic information required for the DBS control and regulatory function and contributes also to the research program of the Division.

Proposed Course of Project: This project will be continued.

Honors and Awards: None

Publications:

Li, C.P., Prescott, B., Eddy, B., Caldes, G., Green, W. R., Martino, E. C., and Young, A. M.: Antiviral activity of paolins from clams. Ann. N. Y. Acad. Sci. 130: 374-382, July 1965.

Serial No. DBS 86
1. Biophysics and Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Sept. 5, 1965 through June 30, 1966

Project Title: Characterization of viruses.

Previous Serial Number: None

Principal Investigator: K. O. Smith, Ph.D.

Other Investigators: J. F. Thiel
W. D. Gehle

Cooperating Units: None

Man Years

Total: 2.75
Professional: 1.75
Others: 1.00

Project Description:

Objectives: (1) To correlate physical and biological properties of viruses.

(2) To develop more specific and more sensitive methods for identifying and classifying viruses.

(3) To study the structure and immunologic reactions of viruses at the molecular level by electron microscopy; special attention is being given to the properties of adeno-associated viruses (AAV) and their interactions with adenoviruses.

(4) To study the adenovirus-cell transforming phenomenon.

Methods Employed: High resolution electron microscopy, virus particle counting, complement fixation reactions, precipitin reactions, agglutination reactions and virus infectivity titrations in tissue cultures are being used to study selected virus-cell systems. Much of this year has been spent developing these techniques so that they can be applied to achieving the objectives listed above.

Major Findings: (1) Detection of an adeno-associated virus (AAV) in a DBS-LBB laboratory strain of adenovirus type-4. Microscopic examination of several adenovirus strains in LBB revealed the presence of high concentrations in one. This adenovirus type-4-AAV mixture has been the subject of the study described below.

(2) Virus purification; density of AAV and adenovirus type 4 in CsCl. Over 75% of adenovirus type 4 particles had a density of 1.34 gm/cm^3 in CsCl. AAV particles varied widely in density, however, many of them having the same density as adenovirus. Separation of adenovirus completely free of AAV by the application of this method only would, therefore, be impossible. The modal density of AAV was 1.37 gm/cm^3, although some particles were as heavy as 1.44 gm/cm^3. AAV particles having a density greater than 1.38 gm/cm^3 were microscopically free of adenovirus particles and were used for antiserum production. About 90% of the adenovirus and AAV CF antigen activity in crude natural mixtures was in the less dense, slow sedimenting "soluble" fractions.

(3) Diameter and fine structure of AAV. The angular, roughly hexagonal outline of AAV in electron micrographs is consistent with its being an icosahedron. Particles averaged 18 $m\mu$ in the smallest dimension and 21 $m\mu$ in the largest dimension. Hypothetical models have been constructed on the basis of information obtained from high resolution electron micrographs. AAV appears to be the simplest possible icosahedron, having a reovirus type, net-like capsid with one hole at each of its 12 vertices. AAV appears to be structurally unrelated to its associated adenovirus.

(4) Resistance of AAV to disruption by detergents. Mixtures of AAV and adenovirus were treated with 12 mg % sodium lauryl sulfate (SLS). Adenovirus capsids were broken into characteristic 1, 3, 7, and 9 capsomer fragments, whereas AAV particles were not disrupted or detectably altered. Purified adenovirus suspensions free of detectable AAV did not yield AAV-like structures when disrupted by SLS. This is further evidence that AAV is structurally unrelated to its associated adenovirus.

(5) Preparation and application of antisera. Rabbit antisera reactive against adenoviruses only and against AAV only have been prepared. These antisera contained no antibody to media or host cells which could be detected in the CF reaction. AAV antiserum has been used to rid a contaminated adenovirus culture of AAV by neutralization. AAV-antibody agglutination reactions have been observed in the electron microscope in a manner which permits visualizing the attachment of γ-globulin molecules upon the viral surface. The number of antigenic sites available for reaction on the particle surface of one AAV strain is estimated to be about 60. AAV appears to be antigenically distinct from adenoviruses in neutralization, agglutination, and CF tests, although both grow to high concentrations in the same cell cultures.

(6) Examination of 13 prototype adenovirus strains for AAV. Adenovirus types 1-12 and type 18 (obtained from the American Type Culture Collection) were passed in HEK and KB cells, then tested by the CF reaction for the presence of AAV antigens. Antisera against one of our own AAV isolates and two antisera against AAV strains isolated by Dr. David Hoggan were used. Only adenovirus type 18 was found to contain AAV. These results were confirmed by electron microscopy. However, the low sensitivity of the CF and electron microscopic methods for detecting AAV suggests that caution be used in interpreting negative results.

(7) Titration of adenovirus type 4-AAV combination in human embryonic kidney (HEK) cells. Limiting dilutions of adenovirus-AAV mixtures required 14-19 days before obvious cytopathic effects could be seen in HEK cells. The infectious virus dose was almost exactly proportional to the time of cytopathic development over a wide dose-response range. All cultures showing cytopathic effects contained adenovirus. Measurable replication of AAV in the absence of adenovirus particles was not observed. We conclude, therefore, that this AAV requires adenovirus as a helper.

Significance to Bio-Medical Research and the Program of the Division: Development of methods for detecting and determining the significance of adeno-associated and other adventitious agents in vaccines is desirable. The AAV-adenovirus system described above has been used to study the capabilities and limitations of several detection methods.

Proposed Course of Project: This project will be continued.

Honors and Awards: None

Publications: None

DCRT
LBBP
LCA
LP
LVI
LVR
CONTRACTS
SUBJECT INDEX
INVESTIGATORS
PUBLICATIONS

Annual Report of the
Laboratory of Blood and Blood Products
Division of Biologics Standards
July 1, 1965 through June 30, 1966
Summary Statements

I. Intramural Research Activities: The research program of the Laboratory of Blood and Blood Products has included studies of the stability of blood products, the development of methods and standards for these products, the investigation of red cell antigens, plasma antibodies, and the clotting and fibrinolytic systems, and the examination of proteins of body fluids. These various fields of interest have as their goal the improvement of procedures used for the control of safety, purity and potency of biological products derived from blood. Thus the projects are directed toward improving existing control tests, developing new ones, obtaining stability data leading to more realistic dating periods, and providing the professional staff with constantly updated information necessary in evaluating new products and procedures.

Basic research in the Laboratory has dealt with various phases of the clotting and fibrinolytic systems. For example, in the course of these studies it was shown that when fibrinogen rich in antihemophilic factor (AHF) was clotted by thrombin, the polymerization of fibrin was accompanied by significant losses of AHF. When glycine ethyl ester was added to the medium at an appropriate concentration, 80 to 90 per cent of the fibrinogen could be clotted with a loss of less than 10 per cent of the total AHF activity.

II. Control Activities: The primary function of the Laboratory is the control of the safety, purity and potency of blood and blood products. Approximately three-fourths of the total time of the Laboratory personnel is occupied by this function. Control activities included the inspection of 172 licensed establishments at a total of 257 locations and the investigation of 4 possible violations of the Public Health Service Act. This required 418 man days of travel. Laboratory personnel performed a total of 25,114 tests on 3014 samples of blood products submitted for release, licensure, or as a result of inspections. License applications for 54 products and 6 establishments were reviewed and forwarded for approval. More than 350 groups of labels and circulars for blood products were reviewed for compliance with regulations. In addition, the development of Tentative Technical Standards for biological products derived from blood constituted an integral part of the Laboratory's control activities.

III. Contract Activities: To supplement the research activities of the Laboratory and yield specific information necessary for the development of regulatory standards, 3 research contracts are in force. The project titles of these contracts are:

1) Plastic-biologics interactions (University of Texas)

2) Influence of various materials on human hemo-antibodies (Baylor University)

3) Standardization of blood collection techniques (Sharpe Research)

IV. Training Program: In support of control functions both laboratory studies and an inspector training program were carried out. The laboratory studies are designed to gain experience and factual information concerning the control testing and properties of new products submitted for license. The training program is designed for the developing of new inspectors and to provide the opportunity for experienced inspectors to discuss interpretations of regulations and problems encountered during inspections.

Serial No. (NIH-DBS) 33
1. Blood & Blood Products
2. Blood & Blood Derivatives
   Diagnostic Reagents
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Preparation, Characterization, Stability and Potency of Blood Products

Previous Serial Number: Same

Principal Investigator: John N. Ashworth, Ph. D.

Other Investigators:
G. Preston Art, B. S.
Marietta Carr, B. S.
Madge L. Crouch, M. A.
Brenda Flam, B. S.
Catherine Knapp, B. S.
Jeanne Lacerte, A. B.
Edward H. Mealey, Ph. D.
Robert M. Pennington, M. S.
Donald L. Powell, B. S.
Rosemary Thorwarth
Donna Williams, B. S.

Cooperating Units: Laboratory of Viral Immunology, DBS (Eva R. Brown)

Man Years: **Patient Days:** None
Total: 4.4
Professional: 2.7
Other: 1.7

Project Description:

Section I. Stability of Blood Products

Objectives: To determine the changes in properties of blood proteins which occur during processing, storage and related treatment.

Methods Employed: a) Samples of Immune Serum Globulin (Human) of various ages and prepared by different manufacturers have been examined by electrophoresis, ultracentrifugation, gel filtration, trichloroacetic acid precipitation and by antibody (measles and poliomyelitis) titrations. (Art & Mealey)

b) To explore methods applicable to the control testing of particle size of aggregated albumin preparations, albumin was heat aggregated in our Laboratory and subjected to particle size measurements. (Art & Mealey)

c) The study of the stability of Blood Grouping Serums at different storage temperatures has been continued, utilizing the methods routinely employed for the control of such serums. (Flam)

Major Findings: a) Results obtained to date (representing 40 samples from 4 different manufacturers) indicate that the amount of trichloroacetic acid soluble material in immune serum globulin increases progressively during storage. Concomitantly, material with a sedimentation coefficient of approximately 3 S appears in these preparations, and the amount of such material increases with time. Starch gel electrophoresis has demonstrated an increase in the number of protein components during the storage period.

b) Differential filtration followed by particle sizing with the Coulter Counter was found to be no more precise than were hemocytometer measurements of aggregated albumin particles.

c) Approximately 1900 potency tests have been performed in monitoring the first year of storage of Blood Grouping Serums. Within the limits of experimental error, no differences in stability have been demonstrated in the serums tested.

Section II. Development of Methods and Standards

Objectives: To develop preparations for standardization of assay methods, to modify existing techniques, to gather information regarding material which comes in contact with blood products so as to provide a basis for future control tests.

Methods Employed: a) To evaluate the level and type of microbial contamination on the outside of blood containers in which inspection samples are received from licensed manufacturers, plate cultures (obtained by using Rodac plates filled with sheep blood agar) were made of the outside of blood containers and of the inside of the shipping containers in which these were sent. (Lacerte & Carr)

b) To develop methods for measuring the physical and chemical interaction of a preservative and surface antiseptic with a plastic which may be used as a packaging material for biologicals, a study of the pilot system, benzalkonium chloride + nylon-6,6, has been undertaken. Coefficients of diffusion, permeation and solubility are determined for varying concentrations of benzalkonium chloride at temperatures of 40° to 70°C. (Powell)

c) By a combination of written inquiry and personal visits, attempts are being made to acquire information on the utilization of plasmapheresis. Data being sought include the number of establishments and individuals involved in plasmapheresis programs, the quantity of plasma obtained by this process, the criteria of eligibility of donors, and the measurements which establishments currently make in order to evaluate the plasma obtained by plasmapheresis. (Crouch)

d) To evaluate the effect of high molecular weight additives on blood typing serums, various concentrations of polyvinylpyrrolidone (PVP) have been

added, in the presence and absence of bovine serum albumin (BSA), to systems containing group 0, Rh_o (D) positive cells and anti-Rh antiserum. Changes in agglutination intensity and titer have been determined. (Thorwarth)

e) Methods of preparation of Reagent Red Blood Cells (Human) were studied, and the stability of such cells prepared in our Laboratory or by licensed manufacturers was determined by serological testing. (Pennington)

f) Work has been initiated with the object of developing a reference anti-human chorionic gonadotropic serum. (Williams & Knapp)

Major Findings: a) Results of 224 plate counts on 87 inspection samples have indicated an average of 6 colonies per plate. Further culturing has demonstrated the presence of the usual air and skin microorganisms but few gram negative bacilli or Streptococcus fecalis organisms.

b) Measurements made thus far on the benzalkonium chloride + nylon-6,6 system appear to confirm the initial hypothesis that the diffusion, permeation, and solubility coefficients depend on concentration, temperature, and time. Other factors that must be considered are the cationic characteristics of benzalkonium and its critical micelle concentration.

c) In various plasmapheresis programs, donors undergoing weekly or biweekly bleeding are providing 750 to 1200 ml. of plasma per week for periods of 1 to 2 years without evidence of depressed plasma protein levels. On the other hand, since plasmapheresis is becoming an increasingly popular source of human blood protein, standardization of current procedures and measurements appears to be necessary.

d) Using group 0, Rh_o (D) positive red cells, it was found that a thousand-fold increase in titer (compared with controls containing no PVP but equivalent levels of BSA) could occur when agglutination was carried out in the presence of 1% PVP and 5% BSA. PVP in the absence of albumin caused no rise in titer. In the presence of 20% BSA, PVP brought about little or no rise in titer, low levels (0.02 to 0.09%) of PVP under these conditions producing an actual decrease. No evidence of non-specificity was obtained. Using a battery of different cells and a so-called "stick" agglutination technique, however, non-specificity was often detected when K_{90} PVP (very high molecular weight) was employed and was occasionally seen when K_{30} PVP (molecular weight 30-40,000) was utilized.

e) Reagent Red Blood Cells obtained from 5 manufacturers proved to be stable throughout their specified dating periods; those obtained from 2 other manufacturers had deteriorated by the end of their dating period. Reagent cells prepared by various methods in our Laboratory are currently being used to study the effects of diluents on antigen stability.

f) Although significant time and effort have been expended, work on a reference anti-human chorionic gonadotropic serum has not yet progressed sufficiently far to permit a report of findings.

Significance to Bio-Medical Research and the Program of the Division: The Laboratory of Blood and Blood Products is responsible for control of the safety, purity and potency of products derived from blood. Such surveillance involves the possession of knowledge regarding the stability of biological products as well as the availability of appropriate control test methods and technical standards. This project fulfills these needs by providing information leading to more realistic dating periods and control test methods.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications: Finlayson, J. S.: Effects of long-term storage on human serum albumin. II. Follow-up of chromatographically and ultracentrifugally detectable changes. J. Clin. Invest. 44:1561-1565, September 1965. (work completed in 1964).

Serial No. (NIH-DBS) 81
1. Blood & Blood Products
2. Coagulation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Clotting and Fibrinolytic Systems

Previous Serial Number: (NIH-DBS) 33

Principal Investigator: David L. Aronson, M. D.

Other Investigators: Russell H. Tomar, M. D.

Cooperating Units: None

Man Years:
Total: 2.1
Professional: 1.7
Other: 0.4

Patient Days: None

Project Description:

Objectives: To purify and characterize components of these systems and to develop an understanding of their chemistry as a basis for more useful assay procedures.

Methods Employed: a) Further attempts have been made to purify human thrombokinase from citrated whole plasma. In addition to those methods already in use, chromatography on hydroxylapatite columns has been employed. Characterization of the reactions during the conversion of prothrombin to thrombin has involved chromatography on DEAE cellulose and disc electrophoresis on polyacrylamide gels. (Aronson)

b) The action of thrombin on antihemophilic factor (AHF) was studied by allowing purified thrombin to act on AHF-rich fibrinogen in a variety of different media. (Aronson)

c) Work undertaken to purify streptokinase has involved salt fractionation, gel filtration, and column chromatography on various adsorbents. (Tomar)

Major Findings: a) Chromatography of human thrombokinase preparations on hydroxylapatite columns has been successful in separating prothrombin from thrombokinase. The latter has then been used as a catalyst to convert prothrombin to thrombin. Examination of intermediates and products formed during the conversion has shown that the reaction is stepwise in nature and follows the same pathway as that observed when bovine thrombokinase or cruder

human systems were used for catalysis. The characteristics of human thrombokinase appear very similar to those of bovine thrombokinase reported by Milstone; moreover, assay systems utilizing Russell Viper Venom strongly suggest identity of thrombokinase and Factor X.

b) When fibrinogen rich in antihemophilic factor (AHF) was clotted by thrombin, most of the AHF activity was lost. Similarly, even in the absence of active thrombin (i.e., after inactivation of the thrombin with diisopropylfluorophosphate) polymerization of fibrin by calcium was accompanied by significant losses of AHF. By adding 0.3 M glycine ethyl ester to the clotting medium, however, it was shown that in the presence of both calcium and active thrombin 80 to 90 per cent of the fibrinogen could be clotted with a loss of less than 10 per cent of the total AHF activity.

c) Starting with a commercially available streptokinase preparation, it has been possible to achieve a 3-fold purification of streptokinase by chromatography on hydroxylapatite. Rechromatography of the appropriate fractions on DEAE cellulose has resulted in a further 3-fold purification.

Significance to Bio-Medical Research and the Program of the Division: Control of the purity and potency of therapeutic blood derivatives used to treat coagulation and thrombotic disorders involves detailed understanding of the mechanisms involved in the clotting and fibrinolytic processes and the chemistry of the *in vitro* assay methods. This understanding is leading to improved validity and precision in control tests.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications: Aach, R. D., and Aronson, D. L.: Association of serum hepatitis with one lot of dried irradiated pooled plasma. J. Am. Med. Assn. 193:17-19, July 1965.

Serial No. (NIH-DBS) 82
1. Blood & Blood Products
2. Coagulation
   Diagnostic Reagents
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Proteins of Body Fluids

Previous Serial Number: (NIH-DBS) 33

Principal Investigator: John S. Finlayson, Ph. D.

Other Investigators: David L. Aronson, M. D.
Robert M. Pennington, M. S.

Cooperating Units: National Institute of Allergy and Infectious Diseases (Dr. Richard M. Asofsky); National Cancer Institute (Dr. Harold P. Morris; Dr. Michael Potter).

Man Years:
Total: 2.1
Professional: 1.2
Other: 0.9

Patient Days: None

Project Description:

Objectives: To complement available data on blood proteins with a reservoir of basic information on the properties of and methods suitable for use on proteins of blood and other fluids; to explore new methods for production, measurement, and investigation of blood group antibodies.

Methods Employed: a) In association with Drs. M. Potter and H. P. Morris of the National Cancer Institute and Dr. R. M. Asofsky of the National Institute of Allergy and Infectious Diseases, the study of proteins of the fluids of laboratory animals was continued. In addition to the physical, chemical, and immunologic methods employed in the past, this year's work on the major urinary protein of mice also made use of tissue incubation and an autoradiographic immunochemical technique. Furthermore, preparative ultracentrifugation in connection with ion exchange chromatography was used to investigate the cause of increased antihemophilic factor activity in the plasma of mice bearing one type of plasma cell tumor. (Finlayson & Aronson)

b) Work on the isolation and study of a human plasma protein with the electrophoretic mobility of γ-globulin was continued, utilizing acid and salt precipitation methods and column chromatography for preparation; analytical ultracentrifugation and electrophoresis as methods of identification and criteria of purity; and disc electrophoresis in polyacrylamide gels and immunodiffusion in agar gels as methods of examination. (Finlayson)

c) The program involving sensitizing mice with Freund's incomplete adjuvant to produce ascites followed by stimulating with appropriate antigens has been continued on a limited basis. Stimulation has been done with group A and B erythrocytes and with blood group specific substance A. (Pennington)

Major Findings: a) Examination of the normal urinary protein of rats of 3 inbred strains and one non-inbred stock showed that, contrary to previous assumptions, a considerable portion of the total urinary protein was of low molecular weight, especially in the males. In the case of male rats of the Buffalo strain, the major urinary protein fraction isolated by gel filtration and two subfractions of it prepared by ion exchange chromatography had molecular weights of the order of 15,000.

Mouse liver (but not the 8 other mouse tissues examined) was found to incorporate C^{14}-labeled amino acids into the major urinary protein complex in vitro. The degree of incorporation was greater in livers from males than from females, and was intermediate in livers from females treated with testosterone. These and previous experiments thus indicate that the major urinary protein complex is synthesized by the liver, released into the plasma, and readily excreted in the urine.

Of the 20 lines of mouse plasma cell tumors tested, the only line that caused an increased plasma level of antihemophilic factor also caused the accumulation of a macroglobulin in the plasma. This macroglobulin was therefore isolated and injected into mice without tumors. Subsequent to the injection there was a 2-fold increase in antihemophilic factor activity which lasted approximately 48 hours; appropriate controls showed no increase.

b) Biophysical, immunologic and chemical data indicate that this protein is identical with β_2-glycoprotein I (β_2-mucoid) described by the Behringwerke group. Preparations which are homogeneous by analytical ultracentrifugation, moving boundary electrophoresis, column chromatography and immunodiffusion show two or three bands by disc electrophoresis. Work is in progress to separate and study these subfractions.

c) Ascitic fluid from mice treated as described above has been harvested in anticipation of comparing antibodies produced by stimulation with red cells with those produced against group specific substance A. Commercially available blood group specific substance B has been shown to be contaminated with group specific substance A.

Significance to Bio-Medical Research and the Program of the Division: The control program of the Laboratory continually requires information regarding the characteristics of proteins and methods used in studying them. The basic research program described above provides the professional staff with a constantly updated pool of fundamental information (including research methodology) necessary in evaluating new products and tests and in discussions with manufacturers.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications: Finlayson, J. S., and Morris, H. P.: Molecular size of rat urinary protein. Proc. Soc. Exptl. Biol. Med. 119:663-666, July 1965.

Finlayson, J. S., Asofsky, R., Potter, M., and Runner, C. C.: Major urinary protein complex of normal mice: origin. Science 149:981-982, August 1965.

Report on Other than Research Activities
Laboratory of Blood and Blood Products
Division of Biologics Standards
July 1, 1965 through June 30, 1966

Under the basic authority of the biological products control provisions of the Public Health Service Act, the Laboratory of Blood and Blood Products of the Division of Biologics Standards has carried out its primary function of controlling the safety, purity and potency of blood and blood products.

1) Table I and Table II indicate some of the functions performed as part of the Laboratory's control activities.

a) Table I shows that 427 man days were spent in performing investigations of possible violations of the Public Health Service Act and on site inspections of foreign and domestic licensed establishments as required by the regulations.

At the beginning of the period covered by this report there were 12 qualified inspectors and 4 trainees available to perform this work. Since that time the Laboratory lost the services of 3 inspectors and 2 inspector trainees and added 2 inspectors and 5 inspector trainees.

b) Table II shows the control testing that has been carried out on the blood and blood products produced by 172 licensees operating 257 principal locations and 247 blood donor stations and subsidiary facilities. Each year these establishments produce in excess of four million units of Whole Blood alone. The control testing of the 3014 lots submitted for release, licensure or as a result of inspections included 25,114 physical, chemical, and immunological tests, as well as the review of manufacturers' protocols necessary as a basis for appropriate action. The tests performed can be divided as follows: 9296 for specific purity, 4534 for specific safety and 11,284 for potency.

c) The Laboratory has carried on an inspector training program. This program takes the form of semi-weekly meetings of inspectors and inspector trainees. Detailed reviews of existing regulations are presented and discussions of interpretations of regulations and the problems encountered during inspections are held. From time to time detailed presentations of other pertinent subjects are given by members of the staff. This year such presentations have included a review of the serological tests for syphilis; the relationship of hemoglobin, hematocrit and specific gravity; pyrogen and sterility testing of biological products; and cold ethanol fractionation of human plasma. In addition, drafts were completed for revisions of establishment and product license application forms, and five committees were formed to update Minimum Requirements for Blood Grouping and Typing Serums, to provide knowledge of current state regulations on blood and to study the consistency of the Laboratory's policy on the responsibilities of the licensed establishment.

2) Associated with the control testing program were the following:

a) 54 product license applications and 24 amendments to license applications were reviewed and recommended for approval. Six establishment license applications were reviewed and the establishments recommended for license. At the present time there are 18 establishment and 34 product license applications pending. There are 17 amendments to currently effective license applications being considered.

b) 379 groups of labels and circulars for blood products were reviewed for compliance with the regulations.

c) Tentative Technical Standards for the following products have been prepared and circulated for comment:

Radio-Iodinated (I^{131}) Serum Albumin (Human)
Radio-Chromated (Cr^{51}) Serum Albumin (Human)
Aggregated Radio-Iodinated (I^{131}) Albumin (Human)
Antihemophilic Factor (Human)

d) The Laboratory maintains serological information about a donor panel representing special blood phenotypes. A member of the Laboratory staff draws blood from 15 to 20 such donors each week to meet the needs of the serum test unit for fresh test cells. The serological data concerning each panel member as well as each blood donor at the Clinical Center Blood Bank has been transferred to a Termatrex(R) catalog system which makes the accumulated information rapidly accessible. Thus the Laboratory has access to the entire donor force of the Clinical Center Blood Bank as a possible source of test cells.

e) The Laboratory has continued to distribute a preparation of dried, pooled plasma to laboratories interested in standardizing the assay for antihemophilic factor. This preparation has found considerable acceptance as an "informal international standard."

3) To supplement the research activities of the Laboratory and to yield specific information necessary for the development of regulatory standards, 3 research contracts are in force. The project titles of these contracts are:

a) Plastics-biologics interactions (University of Texas)

b) The influence of various materials on human hemo-antibodies (Baylor University)

c) Standardization of blood collection techniques (Sharpe Research)

The contract involving horse anti-human protein serums was completed. In support of this contract samples from 29 production bleedings were clarified, filtered under sterile conditions and filled prior to testing. Double gel diffusion and immunoelectrophoresis were performed on representative samples from each bleeding.

Summaries of Certain Control Activities
Laboratory of Blood and Blood Products
Division of Biologics Standards
July 1, 1965 through June 30, 1966

TABLE I

CONTROL ACTIVITIES REQUIRING TRAVEL STATUS

Type of Activity	Locations Inspected	Investigations Conducted	Contracts in Effect	Man Days of Travel
Annual Inspections	257			402
Investigation of Possible Violations		4		16
Supportive of Contracts			4	9
				427

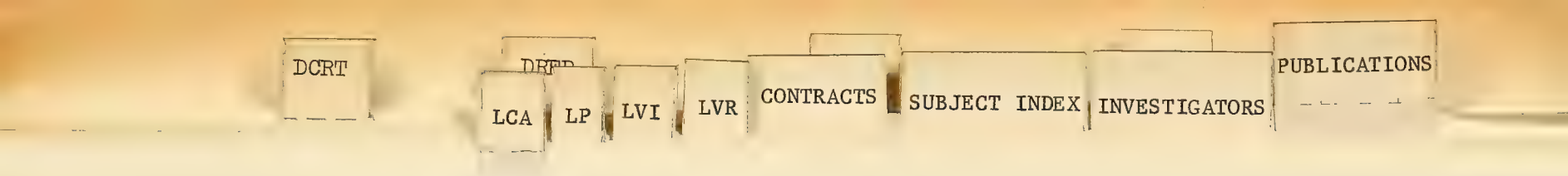
Summaries of Certain Control Activities
Laboratory of Blood and Blood Products
Division of Biologics Standards
January through December 1965

TABLE II

CONTROL TESTING OF BLOOD AND BLOOD PRODUCTS

Type of Product	Inspection Samples	Number of Lots Tested			Number of Tests
		For License	For Release	Total	
Blood Derivatives	15	24	966	1005	2892
Diagnostic Serums	364	17	1122	1503	12,878
Reagent Red Blood Cells	42	11		53	4,810
Whole Blood Plasma Packed Red Cells	415	38		453	4,534
	836	90	2088	3014	25,114

PUBLICATIONS INVESTIGATORS SUBJECT INDEX CONTRACTS LVR LVI LP LCA DCRT


DCRT
LCA
LP
LVI
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PUBLICATIONS

Annual Report of the
Laboratory of Control Activities
Division of Biologics Standards
July 1, 1965 through June 30, 1966

Control of Biological Products and Other Related Activities

Previous Serial Nos.: DBS 34 through 40

Senior Staff: John C. Wagner, Sc.D.
Robert W. Kolb, M.S.
E. B. Seligmann Jr., Ph.D.
Harry W. Burruss

Supporting Staff: Harry T. Aylor
B. J. Baughman (O.D. Assigned)
W. H. Berkeley
Pinya Cohen, Ph.D.
R. D. Crossley II (O.D. Assigned)
Miss Jane Farber
Vernon J. Fuller, Ph.D.
Otis L. Green
W. S. Hunter
J. C. Jenkins
E. C. Russell
W. S. Smith

I. Mission

The Laboratory of Control Activities has prime responsibility within the Division for performing and coordinating the testing conducted on biological products to determine their conformance to standards of safety, purity and potency. The laboratory is composed of three sections: Control Testing, Pyrogens, and Reference Standards; and an administrative technical staff which perform the voluminous task of reviewing manufacturers' product protocols, and coordinating, compiling and filing a major portion of the data of manufacturers submitted to the Division in connection with these control activities.

To fulfill its primary responsibility the Laboratory has the following functions:

(a) To determine whether each lot of a licensed biological product meets prescribed standards of safety, purity and potency and to exercise the necessary control actions culminating in either official release or rejection of the material intended for sale by the manufacturer in interstate commerce. Such action is based on the results of a detailed review of the manufacturers' processing and testing data as reported in manufacturing protocols, testing data compiled within the Division, and any other available source of information relating to the safety, purity or potency of the product.

(b) To develop, maintain and distribute material biological standards, references, control materials and reagents necessary for the testing of products to determine compliance with standards. A culture collection or deposit is also maintained to support the technical effort of the Division as well as to support the control activities as they affect the licensed manufacturers.

(c) To maintain under review published standards, minimum requirements, technical memoranda and other guidance issued by the Division to manufacturers concerning licensable biological products, to develop or propose revisions in regulations when indicated, and to prepare technical specifications and other regulatory procedures for new products as they are developed.

(d) To maintain a close liaison and working relationship with other laboratories of the Division and where indicated with the other Institutes of N.I.H. as well as other outside agencies to insure a continuous flow of knowledge and technical information deemed vital for the proper exercise of control measures of both those products already licensed and new products being developed for license.

(e) To participate in and perform annual inspection of designated licensed establishments in accordance with specified procedures and specific objectives as prescribed by law.

(f) To participate in and give technical support to investigations performed by the Division in connection with Public Health Service Act.

(g) To participate in the review of applications and reports of manufacturers and their products to determine eligibility for license.

(h) To participate in and give technical support to personnel training programs organized within the Division of Biologics Standards.

II. Product Testing, Protocol Review, Records

Individual lots of licensed products are controlled by the Division through an intricate system of evaluation and release utilzing data submitted by the manufacturer and that developed by the testing program of the Laboratory of Control Activities and other testing areas of the Division. All testing is directed toward the objective of making assurance that products meet prescribed standards of safety, purity and potency. The types of products controlled include antitoxins and therapeutic immune serums, blood and blood derivatives, bacterial vaccines, toxins and toxoids, multiple antigens, viral and rickettsial vaccines, diagnostic substances for dermal tests, antivenins, venoms, streptokinase-streptodornase, and allergenic extracts.

Table I presents a summary of 9,595 tests performed by the laboratory for a 12 month period (April 1, 1965 through March 31, 1966) in making the determination that products meet prescribed standards.

TABLE 1

Samples Tested	Number of Tests Performed				
	Sterility	Safety	Potency	Purity	Total
Products for Release	3,380	947	1,099	787	6,213
Inspection	570	76	10	37	693
Cooperative Service	1,878	15	50	506	2,449
Reference and Standardization	0	0	201	0	201
Complaint	17	2	20	0	39
Totals	5,845	1,040	1,380	1,330	9,595

Results of these tests served in part as a basis for concluding that products could or could not be released by the manufacturer for market. Also submitted to the laboratory for review were protocols of the manufacturer showing details of testing procedures and results of all tests performed to demonstrate that each lot of product met prescribed standards. For the year, 3,559 lots of products together with their manufacturing and testing protocols were submitted by manufacturers to the Division for consideration. Of these 3,438 lots were released, 66 lots were rejected by the DBS and an additional 55 lots were withdrawn by the manufacturers without release action being taked by the Division. A summary of these data broken down according to product groups is presented in Table 2.

TABLE 2

Product Group	Lots Released	Lots Rejected	Lots Withdrawn	Totals
Antitoxins	40	0	1	41
Therapeutic Serums	20	0	1	21
Blood and Blood Products	855	2	7	864
Bacterial Vaccines	113	1	3	117
Toxoids and Toxins	74	0	0	74
Multiple Antigen Preparations	139	7	6	152
Viral and Rickettsial Vaccines	785	31	13	829
Diagnostic Substances for Dermal Tests	199	0	0	199
Diagnostic Substances for Laboratory Tests	1,066	25	24	1,116
Miscellaneous	147	0	0	147
Totals	3,438	66	55	3,559

In connection with release activities, the laboratory also prepared the product release actions, maintained a permanent record and filing system of all manufacturers' protocols and test data in support of each action, and maintained refrigerated storage of representative samples along with appropriate records for future reference on all biological products submitted to the Division. In all these activities the laboratory continues to re-evaluate the methods and operations relative to the establishment of more efficient control measures.

III. Material Standards

An integral part of the system of manufacture and control of biological products is the application and use of material standards. By the use of such standards potency of a product can be determined and

standardized so as to give increased assurance of consistency from lot to lot. It is the function of the standards program of the laboratory to provide these standards and reference preparations so that the control program of the Division as well as manufacturers and others engaged in testing may have available the tools to calibrate and evaluate products. It is largely through use of these preparations that those portions of the biologics control regulations relating to potency can be applied.

In order to maintain an adequate supply of material references and standards it was necessary to prepare and calibrate during the past year the following lots of materials: 16 antitoxins, 4 serums, 11 vaccines and 4 toxins. A total of 344 tests was required to complete a satisfactory standardization of these materials. These tests included such procedures as flocculation reactions, animal protection tests, animal titrations, neutralizations and assorted *in vivo* and *in vitro* tests on specific specialized materials.

The technique of freeze-drying is a key procedure applied in the preparation of standards and reference materials whenever possible in order to increase assurance of stability over long periods of storage. The following numbers of ampules of various preparations were dried during the year:

Cultures	1821	ampules
Serums	30	"
Vaccines	1016	"
Viruses	762	"
Toxins	838	"
Total	4467	ampules

The following standard and reference preparations, comprising a total of 899 separate shipments were distributed to manufacturers, P.H.S. and state health laboratories, universities, foreign manufacturers holding U.S. licenses and qualified foreign laboratories (primarily through the World Health Organization).

Antitoxins	490
Serums	2711
Vaccines	2433
Toxins	396
Bacterial & Viral Cultures	1142
Allergens	60
Total	7232

A culture collection of etiological agents, i.e. viral and bacterial organisms, used in the manufacturing and testing of biological products, was maintained. Approximately 80 different standards, references, and related control preparations are maintained for distribution.

IV. Related Activities

In addition to the effort outlined above, the laboratory staff also participated during the year in a number of ancillary activities directly related to control which accounted for a substantial segment of its working time.

(a) At least one professional person (as a representative of the control laboratory interest) participated as a member of each DBS committee appointed to review, evaluate and recommend appropriate action with respect to new or revised product and establishment license applications on all biological products presented to the Division, except blood and blood derivatives in which case the participation of the Laboratory is liaison with the Laboratory of Blood and Blood Products. Such review encompassed detailed scrutiny of manufacturers' production protocols for scientific accuracy and conformance to prescribed standards, testing of the products, inspection of manufacturing facilities, preparation of reports, etc. Fifty-one product licenses and six establishment licenses were processed during the year and control testing of the products submitted in support of these applications were performed in the laboratory. Reports and recommendations were made for each action.

(b) The senior laboratory staff participated in thirty-five of the on-site inspections required to be performed annually on all licensed establishments and in three pre-license inspections routinely performed on each establishment prior to recommending issuance of license. Approximately 85 man days of work were consumed during the year in this effort.

(c) Staff participated in the Division program concerning the conduct of investigations of alleged or suspected violations of the Public Health Service Act with respect to biological products. Such effort involved the collection and assembling of evidence and technical information for use in potential legal actions brought against violators of the Act. One member of the staff served as a case supervisor in a Division investigation of an unlicensed biological manufacturer found violating the Act. Legal action was initiated and completed during the year.

(d) Numerous members of the staff participated in testing, research, writing and other control activities by which formulation and revision of regulations and additional standards for biological products were effected. Documents of various kinds were drafted for Diphtheria Toxin for Schick Test, Smallpox Vaccine, Typhoid Vaccine, Dating recommendations for licensed biological products, review of WHO requirements for BCG Vaccine, review and evaluation of the International Old Tuberculin Standard.

(e) The staff cooperated with other P.H.S. laboratories, manufacturing establishments, domestic and foreign government agencies interested in testing or evaluating various unlicensed, exotic or unusual biological or pharmaceutical preparations. During the year twenty-two visiting scientists from domestic and foreign public health laboratories and manufacturing establishments were given technical orientation and training in techniques of biological control testing and standardization used in the Division.

(f) The Laboratory participated in inspectors training courses conducted within the Division and provided technical and other assistance in the development of an inspectors training course sponsored by the Division in conjunction with the FDA and University of Rhode Island.

V. Research

A substantial applied research program was conducted during the year for the purpose of solving a variety of problems associated with various disease entities in which the laboratory has product interest. Brief summaries of each of these studies are given below.

(a) Clostridium botulinum Toxin and Antitoxin - The testing program on products manufactured from these materials has a long history of difficulty primarily because of unavailability of a satisfactory stable toxin. Progress is being made in this area, however, and improved potency tests for Botulism Antitoxin Types A, B, C and E are being developed. It is anticipated that revised testing recommendations will result from these studies. A satisfactory Type E toxin has been prepared and a neutralization test is in an advanced stage of development. Concurrently a lot of Botulism Antitoxin Type E is being standardized and is intended to serve eventually as the U.S. Standard for this type of product.

(b) Influenza Virus Vaccines - One of the factors which has been suggested as a possible cause of the reactive properties of influenza vaccines when administered to man has been the presence of varying numbers of dead bacteria in the viral product. An attempt was made to determine if any such correlation could be established by pyrogen testing a number of licensed commercial vaccines in rabbits and simultaneously determining bacterial count by the counting chamber technique.

After studying thirty-four lots of vaccine it was concluded that no such correlation could be demonstrated in rabbits. A report summarizing these findings was prepared.

(c) Coral Snake Venom and Antivenin - Standards suitable for the control of the manufacture of coral snake antivenin are being developed. In the course of this work the immunological and serological properties of the venom and the immunologic response of various species of animals to hyperimmunization are being studied. Methods have been developed for producing high titer neutralizing antibody in rabbits and goats. Techniques of plasmapheresis similar to that in human bleeding programs are being tried for the first time on hyperimmunized goats, and the effects of these repeated bleedings on antibody levels and other blood parameters are being studied. To date no demonstrable adverse effects have been observed and the technique is considered quite satisfactory.

An unusual observation has been made in connection with these studies in that the coral snake venom apparently hemolizes red cells of certain animal species. The significance of this property has not yet been evaluated beyond the suggestion that perhaps a hemolytic factor is being demonstrated in the venom in addition to what has heretofore been

considered to be exclusively neurotoxic activity. A contract which provides coral snake venom for this project is also producing data for the first time on the venom yields from coral snakes of different sizes and species.

(d) Smallpox Vaccine - During the past year the World Health Organization has adopted and published international standards for smallpox vaccine employing the chorioallantoic membrane (CAM) pock counting method as the preferential official procedure for determining potency. This test is in contrast to the rabbit scarification (RS) method which is still the only test accepted in the U.S. for evaluating potency of lots of vaccine. World Health Organization has set the potency standard of the international reference at 1×10^8 PFU/ml, with no confidence limits stated.

It is anticipated that the DBS will have a need in the future for data for establishing a correlation between the CAM and RS tests. Therefore an extensive comparative testing program has been in progress to evaluate the two methods. To date the following observations and determinations have been made.

1. The potency of the U.S. Reference Lot No. 2, when tested by the CAM test in 61 titrations, has been determined to be $10^{8.07}$ PFU/ml, with a standard deviation of $\pm$ 0.20. Where tested by the rabbit scarification procedure, the relative potency of the international reference when compared to the U.S. reference was 0.92. These data have been interpreted to indicate that, for purposes of testing the U.S. and international references are of essentially equivalent potency.

2. With the exception of smallpox vaccine samples of one manufacturer out of eight whose products were tested, the comparative RS and CAM assay procedures on 60 vaccines yielded potency values with the two techniques which were in relatively close agreement.

After resolving the reason for differences in potency values obtained for the two test systems of one manufacturer's product, we hope to be able to determine if the CAM test can be accepted by the Division of Biologics Standards as an alternate to the RS test for purposes of evaluating potency of commercial smallpox vaccine products.

(e) Sterility Testing - Regulations require of manufacturers that, in performing their sterility tests on products, appropriate controls will also be included which will demonstrate that the media will support growth of bacterial contaminants and thus that the tests are valid. Therefore the growth-promoting properties of media used to detect viable anaerobic bacteria in a sample are also tested using suitable known anaerobic cultures. Manufacturers who have isolation facilities, where spore-forming organisms can be handled safely, generally use clostridia as test organisms. However, where isolation facilities are not available, a manufacturer is not permitted to introduce spore-forming organisms into the product-manufacturing establishment.

A project was therefore initiated to provide a non-spore-forming anaerobe, fastidious in its growth requirements and at the same time suitable for use as a control in testing the growth-promoting qualities of Fluid Thioglycollate Medium required in the official sterility tests on biological products.

Two species of Bacterioides (ovatus and vulgatus) have been found which appear to have the desired characteristics. Both species have been determined to be more fastidious in their nutritional requirements than are Clostridium tetani and Clostridium feseri. Additional data are being collected in order to develop eventually a satisfactory testing procedure for screening sterility test media.

(f) Toxoplasmin: In cooperation with the Laboratory of Bacterial Products (See DBS Serial No.103,p.26) studies were continued with an objective of developing an acceptable in vivo laboratory test procedure for standardizing the potency of Toxoplasmin skin test antigens. A procedure has been developed and published whereby toxoplasmin preparations of egg tissue and mouse tissue origin can be assayed in sensitizied guinea pigs and an index of potency determined. The test is still not acceptable as a routine procedure however and needs refinements. It has been observed that antigens, although satisfactory as a diagnostic skin test antigen in man, nevertheless are not sufficently concentrated to yield skin reactivity consistently in all hypersensitized guinea pigs. It is felt that this limitation can be overcome and that the test will become applicable.

(g) Tetanus Antitoxin, U.S. Standard - The U.S. Standard Tetanus Antitoxin is a dried globulin material prepared in 1907 from horse serum. This material has been used for many years as a primary U.S. Standard from which diluted liquid preparations are made periodically for distribution in tetanus antitoxin testing programs. The supply of this globulin material is now practically depleted so that a new antitoxin standard must be selected which will prove to be a suitable replacement.

A test method has been developed for the determination of the protective unitage of antitoxin in mouse tests. This has proved to be sufficiently consistent and sensitive to detect five-ten thousandths of a unit difference in standard antitoxin per mouse dose. The L+/100 dose of toxin was ascertained by combining it in varying amounts with 0.01 unit of standard antitoxin.

Three different lots of dried tetanus antitoxin have been screened in preliminary tests and the two which produced consistent results in a limited number of animal tests at the L+/100 dose level of antitoxin are being studied further as candidates for adoption as the standard.

Additional in vivo and in vitro tests have been performed in which a solution of polyvinylpyrrolidone (PVP) has been added, to test the hypothesis that it might alter the endpoint, avidity, stability or

reaction time of these tests. It has been reported that this material will alter the titers of blood-typing sera. It was thought therefore that it might also improve the sensitivity of the avidity, neutralization, and flocculation tests on antitoxins. It has been observed that a six percent solution of PVP substituted as diluent in flocculation tests with antitoxin will reduce the reaction time without changing the relative unitages of the antisera. Thus far, PVP has not been shown to influence in vivo results. These studies are continuing.

(h) Typhoid Vaccine - In anticipation of the interest that will probably be developing in an acetone-killed typhoid vaccine over the present heat-killed phenolized product, studies have continued during the year on development and standardization of an acetone-killed and dried standard. Candidate Lot No. 6A is being tested extensively and has been shown thus far to be acceptable. Results of stability tests on this material by mouse potency assay have indicated no loss after 24 months of storage at 4°C and 22°C. Samples stored at 37°C showed an approximate 30% loss in potency after 24 months. These data will be extremely valuable along with those collected by manufacturers in determining, later, the dating period and storage recommendations for the acetone-killed typhoid vaccine product.

Publications and Technical Reports

1. Chaparas, S.D., Fuller, V.J., and Kolb, R.W. A Laboratory Procedure for Determining the Potency of Toxoplasmins for Skin Testing. Proceedings of the Society for Biology and Medicine 121 (3): 734-739, 1966

2. Seligmann Jr., E.B., and Berkeley, W.H. Direct contact tray assembly for freeze-drying. Appl. Microbiol., 13: 830, Sept. 1965

3. Technical Report - Burruss, H.W., Farber J.F., Seligmann, E.B., Kolb, R.W. and Wagner, John C. Pyrogen and Other Studies on Influenza Virus Vaccines 1965-66 Formula, November 3, 1965.

4. Technical Report - Fuller, V.J. and Kolb, R.W. Correlation of the Rabbit Scarification and Chorioallantoic Membrane Potency Assay Methods for Smallpox Vaccine. April 8, 1966.

5. Technical Report - Barile, M.F. and Kolb, R.W. Summary Report on Diphtheria Toxin For Schick Test. December 1, 1965.

DCRT
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Annual Report of the Laboratory of Pathology
Division of Biologics Standards
July 1, 1965 through June 30, 1966

The Laboratory of Pathology has now completed its first full year as a separate entity. Until this year, the Laboratory's largest project has been the performance of neurovirulence tests of lots of Poliovirus Vaccine, Live, Oral. However, with the great decline in poliomyelitis in the United States and in the world, the number of lots submitted for testing has markedly decreased. As a result, the laboratory has been able to turn its attention to other problems related to the pathogenesis of infectious diseases and their prevention or control.

In addition to its own research and control activities, the Laboratory has had an active collaborative research program with members of the staffs of the Laboratory of Viral Immunology, the Laboratory of Virology and Rickettsiology, the Laboratory of Bacterial Products, the Laboratory of Biophysics and Biochemistry, the Laboratory of Control Activities within the Division and with several members of other institutes as well.

The two sections within the Laboratory work closely together and indeed each complements the activities of the other.

1. The Section of Animal Testing has the largest facility for monkeys within the Division. The Section is under the general supervision of a veterinarian, Dr. Amos E. Palmer, who has had wide experience in the care of primates. Under his direction, a program of breeding has been undertaken. This has resulted in the birth of 12 baby monkeys of which 3 were rhesus and 9 were stump-tailed. Studies in artificial insemination are in progress which may result in an increased birth rate. These newborn animals are being used by the staff members of the laboratory in its studies of infectious diseases with the hope that newborn primates may have a diminished immunologic response similar to that of newborn rodents. (Proj. No. 96, p. 109)

In collaboration with the Laboratory of Viral Immunology, various strains of rubella virus have been studied in monkeys. This virus has not been shown to be neurotropic despite its ability to infect and to be recovered from cerebrospinal fluid and blood and to give an antibody response. (Proj. No. 44, p. 101)

Several secondary seed lots of Yellow Fever Vaccine have been studied in rhesus monkeys as well as the primary seed lot. Basic information has been obtained concerning the primary seed lot so that, in the event of the need for a new seed lot, a comparison can be made between the two. This study was carried out with the Laboratory of Control Activities. (Proj. No. 44, p. 103)

In collaboration with the Laboratory of Virology and Rickettsiology a study of the pathogenesis of Typhus Fever in rhesus and vervet monkeys

was undertaken. In addition, with the staff of this laboratory, attempts to elucidate the cause of an epidemic hemorrhagic disease of monkeys have been continued. (Proj. No. 44, p. 102)

The use of monkeys to study various toxins has been implemented. It is hoped that since these animals are so similar to man that evaluation of these toxic effects and their prevention may be elucidated. (Proj. No. 95, p. 108)

2. The Section of Histopathology has on its staff an anatomist who was awarded a Ph.D. degree in February, 1966. Dr. Ronald Clark is well-versed in histology, cytology and embryology and has become expert at interpretation of abnormal histologic findings and has developed several independent and collaborative research projects. His studies of the neuro-anatomical development of various animal species and the relationship of this development to the ability of animals to develop allergic encephalo-myelitis or of the tissues to produce such an encephalitis will be expanded during the coming year. Studies on the relationship of neuroanatomical structure to the pathogenesis of diseases induced by neurotropic viruses are continuing. It is hoped that these studies may lead to more sensitive safety tests for vaccines. (Proj. No. 45, p. 105)

In collaboration with the Laboratory of Bacterial Products, studies on hypersensitivity especially to tuberculin or B.C.G. vaccine and of the effects of mineral oil adjuvants have continued. (Proj. No. 44, p. 102)

Under the direction of the Chief, a large part of the research efforts of the Laboratory involve studies of oncogenic viruses and have been concentrated during this year on elucidating the mechanism of interactions of two oncogenic viruses (namely, SV40 and oncogenic adenoviruses) on each other and on the host. (Proj. No. 44, p. 101)

It is expected that the activities of this laboratory will greatly expand as collaborative research projects, contracts programs and control activities of the Division require more and more animal studies and histologic evaluation.

Control Activities

The scientific program of the Laboratory of Pathology is a close blending of research and control activities with each function being dependent on the other for the development of new activities and the refinement of the old ones. Control testing is performed on Poliomyelitis Vaccine, Poliovirus Vaccine, Live, Oral and Measles Virus Vaccines in order to assure the safety of these products. Each section in the laboratory participates in these tests; one to perform the test, observe the animals and provide the tissues; the other to process the tissues and interpret the results.

Control neurovirulence testing of Poliovirus Vaccine, Live, Oral is most critical and at present all vaccine lots received are tested. These

lots are tested in comparison with a reference vaccine so that the established standards may be maintained.

A total of 23 vaccine lots have been tested in monkeys during this year; 5 were Poliovirus Vaccine, Live, Oral, 5 were Poliomyelitis Vaccines and 13 were Measles Virus Vaccines. A total of 1030 monkeys have been used in this program. (Previous Proj. Nos. 41, 42, 43)

The Histopathology Section prepared 38,257 slides during the year of which 24,682 were hematoxylin and eosin or special stains, 8,652 were gallocyanin stains and 4,923 were blood smears. The Section has developed the ability to do hematologic studies on laboratory animals as needed. It is planned to expand this capability to include other clinical pathology studies as required by the investigators in the Division.

Serial No. DBS 41
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Developmental studies of Neurovirulence Testing on Poliovirus Vaccine, Live, Oral, in monkeys.

Previous Serial Number: 41

Principal Investigator: Ruth L. Kirschstein, M.D.

Other Investigators: Amos E. Palmer, D.V.M., LP-DBS
Ronald G. Clark, Ph.D., LP-DBS
George Rusten, LP-DBS

Cooperating Units: Serology Section, LVI, screens monkey serums for freedom from antibody to poliovirus.

Man Years:
Total: 6.0
Professional: 0.4
Other: 11.6

Project Description:

Objectives: To develop information concerning the reproducibility of neurovirulence tests of Live Poliovirus Vaccines and to refine techniques used in the test to assure safety of the vaccine.

Methods Employed: Monkeys were inoculated with vaccine or dilutions thereof either intrathalamically or intraspinally, and observed for evidence of developing paralysis over a period of 17-18 days. The monkeys were then sacrificed and the central nervous system examined histologically for evidence of poliomyelitis. In addition, tissues were saved for virus isolation attempts. These studies were performed as outlined in the PHS Regulations for the Live Poliovirus Vaccines.

Major Findings: Several Type III vaccines were tested repeatedly and the reproducibility of the test system could be demonstrated. The data presented led to development of an international study involving the comparative testing of vaccines of varying degrees of neurovirulence. This study is now in progress.

Significance to Biomedical Research and the Program of the Division: These studies were designed to assure standardization of neurovirulence testing of poliovirus vaccines throughout the world and to assure the safety of such vaccines.

Proposed Course of Project: The international comparative study will continue during the next year. It is anticipated that other such collaborative studies to standardize safety testing of other vaccines will be initiated over the next several years.

Honors and Awards:

Ruth L. Kirschstein appointed as a member of the World Health Organization Expert Group on Requirements for Biological Substances. The report of this group is Technical Report Series No. 323, 1966.

Publications:

Kirschstein, R. L. and Clark, R. G.: Reproducibility of Neurovirulence Tests. Presented at the Conference on Testing for Neurovirulence of Virus Vaccines sponsored by the Permanent Section of Microbiological Standardization, International Association of Microbiological Societies, Munich, Germany, August, 1965 (in press).

Kirschstein, R. L.: Value of Neurovirulence Tests for Attenuated Vaccine Assessment. Presented at the Conference on Testing for Neurovirulence of Virus Vaccines sponsored by the Permanent Section of Microbiological Standardization, International Association of Microbiological Societies, Munich, Germany, August, 1965 (in press).

Serial No. DBS 44
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Pathogenesis of Infectious Diseases.

Previous Serial Number: 44

Principal Investigator: Ruth L. Kirschstein, M.D.

Other Investigators:
Alan Rabson, M.D., NCI
Ronald Clark, Ph.D., LP-DBS
Amos E. Palmer, D.V.M., LP-DBS
Philip McGrath, LP-DBS
Elizabeth Peters, LP-DBS
Harry Meyer, M.D., LVI-DBS
Paul Parkman, M.D., LVI-DBS
Samuel Baron, M.D., NIAID
K. K. Takemoto, Ph.D., NIAID
Joseph O'Malley, M.D., LVR-DBS
Harold Baer, Ph.D., LBP-DBS
Sotiros Chaparas, Ph.D., LBP-DBS
Lewellys F. Barker, M.D., LVR-DBS
Carolyn Hardegree, M.D., LBP-DBS
Michael Barile, Ph.D., LBP-DBS
Edward Seligmann, Ph.D., LCA-DBS
Samuel Formal, Ph.D., WRAIR

Cooperating Units:
Pathologic Anatomy Branch, NCI
Laboratory of Pathology, NCI
Laboratory of Biology of Viruses, NIAID
Laboratory of Viral Immunology, DBS
Laboratory of Virology and Rickettsiology, DBS
Laboratory of Bacterial Products, DBS
Laboratory of Control Activities, DBS
Walter Reed Army Institute of Research

Man Years:
Total: 6.0
Professional: 0.8
Other: 5.2

Project Description:

Objectives: Experimental infectious diseases are studied as to pathogenesis in the hope that this may further elucidate the pathogenesis of infectious diseases in man and lead to their prevention.

Methods Employed: Infectious agents and related substances or substances causing an immunologic response are injected into a variety of animals when newborn or adult and gross autopsies and histologic examinations are performed at timed intervals so that the development of the disease process and the pathologic changes may be studied.

Major Findings: (1) (With Dr. Rabson and Mrs. Peters) Studies on the comparative oncogenic effects of polyoma virus strains, simian virus 40 and adenoviruses are continuing. It has been found that polyoma virus produces tumors only in mice, hamsters and mastomys, SV40 in hamsters and mastomys, adenoviruses in hamsters, mastomys and certain inbred strains of mice. The oncogenic potential of these viruses can be enhanced by decreasing the immunologic capability of these animals. This can be accomplished primarily by performing thymectomies on newborn animals.

In addition, studies of a "hybrid" virus of adenovirus 7 and SV40 (strain E46) indicate that the genome of either the adenovirus portion or the SV40 portion have specific oncogenic effects under varying conditions.

(2) (With Dr. Rabson and Mrs. Peters) Studies of cultured cells from lymphomas of the "Burkitt" type and of viruses found by others to be associated with them are in progress.

(3) (With Dr. Rabson) Studies of the ability of the oncogenic viruses to "transform" hamster cells and monkey cells in cell cultures are continuing. The cellular transformations have characteristics of malignancies and have varying growth potential when inoculated into animals depending on the species. Attempts to alter this growth potential are underway.

(4) (With Dr. Takemoto) Studies on the pathogenesis of tumors produced by clones of various SV40 plaque strains indicate that various plaque sizes are of different virulence and oncogenic potential.

(5) (With Drs. Meyer, Parkman and Palmer) Studies of the pathogenesis of rubella virus infection in monkeys are in progress. To date the virus has been found to multiply in the monkey but not to produce any pathologic alterations. Investigation of communicability of various strains of rubella virus from monkey to monkey is underway.

(6) (With Drs. Hardegree and Barile) Studies of the pathologic reaction in muscles of monkeys injected with tetanus toxoid emulsified in mineral oil adjuvant reveal that the emulsion tends to localize in pockets throughout the muscle and to cause severe reactions in the muscle as long as 6 months after inoculation. It appears that this material may remain

in the muscle forever and continue to act as an irritant. Studies of other adjuvants are planned so that information will be available when they are proposed for use.

(7) (With Dr. Clark) Studies of the sensitivity of finite neuroanatomical structures to virulent and attenuated polioviruses are in progress.

(8) (With Dr. Clark) Studies on the comparative sensitivity of intraspinal and intrathalamic routes of inoculation of polioviruses of different virulence are in progress.

(9) (With Mr. McGrath) Studies of the histology of various viral induced tumors using thin sections (1 micron) and electron microscopy are in progress.

(10) (With Dr. Chaparas) Studies of the development of antibody-producing cells in the lymph nodes of guinea pigs required the use of specialized stains. These studies are complicated by the natural flora of the animals which alters the production of antibodies.

(11) (With Dr. Baer) Studies of the skins of guinea pigs injected with various derivatives of poison ivy extract are underway to determine histologic differences between toxicity and sensitization.

(12) (Drs. Palmer, Baer and Chaparas) Studies of the production of antibodies to Mycobacterium tuberculosis are in progress. Monkeys have been inoculated with BCG, and serum samples are being collected from animals reacting positively to tuberculin in the Primate Quarantine Unit.

(13) (With Dr. Baron) Production of circulating interferon in monkeys occurs when virus is inoculated intravenously and viremia occurs.

(14) (With Drs. Palmer and Barker) Studies of the pathogenesis of epidemic typhus fever in monkeys are in progress. Monkeys are susceptible to the disease and the lesions produced are similar to but milder than those in man when the Breinl strain is used. Previous inoculation of the "E" strain is protective against challenge with the Breinl strain.

(15) (Dr. Palmer and Dr. Tauraso) Studies to detect and characterize the agent responsible for an epidemic of a febrile, hemorrhagic disease among monkeys in the quarantine unit in 1965 are continuing. To date, no other host has been found susceptible to the agent.

(16) (With Dr. O'Malley) Studies of an agent (A-1) in small animals are being completed. These studies were designed to determine if these animals develop hepatitis and included histologic examination of needle biopsies of the liver and blood counts as well as virologic and serologic determinations.

(17) (With Dr. Clark and Mr. McGrath) Studies of the ultrastructure of various organs from normal monkeys and of viral-induced tumors in hamsters are in progress.

(18) (With Dr. Seligmann) Secondary and primary seed lots of Yellow Fever Vaccine have been studied in rhesus monkeys so that, in the event that a new seed lot is needed, the neurovirulence level of the vaccine will have been established.

(19) (Dr. Palmer and Dr. Formal) Attempts to develop an immunizing agent against Shigella infections are in progress. Since this is a major cause of fatal enteritis during the conditioning period of monkeys, this is essential.

Significance to Biomedical Research and the Program of the Division: These studies are designed to elucidate aspects of the pathogenesis of infectious diseases in animals so that this model may serve to help provide the means of prevention of such diseases in man.

Proposed Course of Project: It is expected that this program will expand in 1966-67.

Honors and Awards: None

Publications:

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Rabson, Alan S., Kirschstein, Ruth L., and LeGallais, F.: Autologous Implantation of Rhesus Monkey Cells "Transformed" In Vitro by Simian Virus 40. J. Nat. Cancer Inst. 35:981-991, 1965.

Kirschstein, Ruth L., Rabson, Alan S. and O'Conor, Gregory T.: Ependymomas Produced in Syrian Hamsters by Adenovirus 7, Strain E46 ("Hybrid" of Adenovirus 7 and SV40). (30568). Proc. Soc. Exp. Biol. and Med. 120:484-487, 1965.

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Serial No. DBS 45
1. Pathology
2. Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Developmental Structure of the Central Nervous System in Relation to Immunological Response.

Previous Serial Number: 45

Principal Investigator: Ronald G. Clark, Ph.D.

Other Investigators: Philip McGrath, LP-DBS
Joyce Johnson, LP-DBS

Cooperating Units: None

Man Years:
Total: 1.3
Professional: 0.4
Other: 0.9

Project Description:

Objectives: To ascertain the normal developmental processes in the nervous system of laboratory animals and to relate structural alterations of these developmental processes in experimental animals in response to immunologic stimulation.

Methods Employed: In utero and postnatal animals are sacrificed at timed intervals and gross evaluation and histologic examinations are performed to determine the normal development of the central nervous system. The histologic evaluation involves both light and electron microscopy. Experimental animals are studied similarly and microscopic as well as ultrastructural deviations from the normal are noted and evaluated.

Major Findings: Light microscopy studies of the neuroanatomical development of hamsters and rabbits have been completed and extensive studies of other animal species are in progress.

Significance to Biomedical Research and the Program of the Division: These studies are closely related to the problem of post-vaccinal encephalitis and the production of vaccines in animal central nervous system tissue.

Proposed Course of Project: This endeavor will continue on a similar scale with more emphasis being placed on the study of ultrastructural changes in relation to immunological response.

Honors and Awards:

Awarding of Ph.D. degree, February, 1966, George Washington University.

Dissertation:
Ronald G. Clark: Myelinization of the Central Nervous System of the New Zealand Albino Rabbit and the Syrian Hamster.

Publications: None

Serial No. DBS 95
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The Development of Toxic States.

Previous Serial Number: None

Principal Investigators: Ronald G. Clark, Ph.D.
Amos E. Palmer, D.V.M.

Other Investigators: Pinya Cohen, Ph.D., LCA-DBS
Carolyn Hardegree, M.D., LBP-DBS
Roberta Gardner, Ph.D., LBP-DBS
Stephen Potkay, D.V.M., LAB-DRS
Howard Bohner, LAB-DRS

Cooperating Units: Laboratory of Control Activities, DBS
Laboratory of Bacterial Products, DBS
Laboratory Aids Branch, DRS

Man Years:
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: To determine the toxicity of various biological products.

Methods Employed: The materials are inoculated into a variety of animals. Gross autopsies and histologic examinations are performed at timed intervals so that the development of the toxic process and the related pathologic changes can be evaluated. In addition, physiologic, hematologic, chemical and clinical observations are studied.

Major Findings: (1) (With Dr. Cohen) Studies of the pathologic changes induced by the supposed neurotoxic component in coral snake venom are underway.

(2) (With Dr. Gardner and Mr. Bohner) Studies comparing the toxicity of various pertussis vaccines in germ-free mice and conventional mice are in progress.

(3) (With Dr. Hardegree) Studies of the effects of the hemolytic fraction of tetanus toxin in rhesus monkeys have just begun and no effect can be noted as yet.

Significance to Bio-Medical Research and the Program of the Division: The results of this study may be significant in making it possible to reduce the toxicity of biological products used in man.

Proposed Course of Project: This project has just begun and will expand in the coming year.

Honors and Awards: None

Publications: None

Serial No. DBS 96
1. Pathology
2. Animal Testing
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Breeding of Non-human Primates.

Previous Serial Number: None

Principal Investigators: Amos E. Palmer, D.V.M., LP-DBS
William Tullner, Ph.D., LB-NICHD

Other Investigators: Charles Turner, LB-NICHD
Donald Barber, LB-NICHD
Ben Kittridge, LB-NICHD

Cooperating Units: Laboratory of Biology, NICHD

Man Years:
Total: 0.6
Professional 0.1
Other: 0.5

Project Description:

Objectives: To develop techniques to detect the stage of the estrus cycle, period of gestation, and for semen collection, dilution, storage and artificial insemination of monkeys in an effort to increase the efficiency of reproduction.

Methods Employed: Semen is collected by electroejaculation. Liquid portions of ejaculates are evaluated for sperm concentration, motility, and total content. Dilution is performed using one of several extenders and the samples are frozen in liquid nitrogen or used to inseminate females. Cytologic and chemical methods have been used to detect estrus and early pregnancy.

Major Findings: Electroejaculation has regularly produced semen samples of relatively consistent quality. Dilution of the liquified semen permits storage at refrigerator temperature for reasonable periods of time. Freezing in liquid nitrogen permits storage of viable spermatozoa for prolonged periods of time. Losses vary depending on diluent used prior to freezing. Several females have been inseminated without conception to date. As a result of normal breeding procedures 12 infant monkeys have been born in the DBS colony during the past year.

Significance to Biomedical Research and the Program of the Division: This project is an effort to increase the supply of gravid females and/or newborn infant monkeys which are acutely needed for research projects, but are unavailable from commercial sources.

Proposed Course of Project: This project will continue as at present unless developments dictate changes.

Honors and Awards: None

Publications: None

Annual Report of
Laboratory of Viral Immunology
Division of Biologics Standards
July 1, 1965 through June 30, 1966

The Laboratory of Viral Immunology conducts a biologics-related virus research program and assumes other responsibilities having to do with the licensing and control of biological products.

The major research emphasis of the Laboratory during this year has had to do with rubella virus.

There has been a mounting international search for rubella vaccines. In responding to this need the Section on General Virology devised a series of in vitro and in vivo techniques capable of signaling if and when modification of cell culture-propagated rubella virus had occurred. High passage levels of the virus were found to exhibit marked differences in behavior when compared to virulent low passage virus in RK_{13} continuous rabbit kidney tissue cultures. Of even more interest was the observation that the high passage strain evoked the production of increased amounts of interferon in various types of cell cultures.

Realizing the need for in vivo confirmation of this apparent virus modification, the rhesus monkey was used to compare the characteristics of infections produced by the virulent and the modified high passage virus. As reported in this past year's annual report (Proj. No. 49, p.117) the Laboratory of Viral Immunology had established that these animals served as an ideal experimental model since the virologic events associated with virulent rubella virus infections in monkeys closely simulated the circumstances observed in man.

The tests with the high passage strain provided the final clue to the fact that laboratory manipulation had indeed, attenuated the virus. The animals inoculated with virulent rubella virus developed infections characterized by viremia, virus shedding from respiratory and rectal secretions and communicability to uninoculated cage contacts. Monkeys infected with the high passage strain developed antibodies but did not exhibit viremia or significant virus shedding and uninoculated controls remained free of infection.

Acting on these data, the high passage strain was used to prepare an experimental live rubella virus vaccine. This material was inoculated into 8 rubella-susceptible girls under carefully supervised conditions of isolation. None of these children showed any symptoms of illness which could be attributed to the vaccine, yet all developed neutralizing antibodies indicative of immunity. Viremia was not detected. Although small amounts of virus were obtainable from the pharynx of several vaccinees, the material was not communicable, since none of 8 rubella-susceptible intimate contacts were infected.

A larger clinical study just completed confirmed these preliminary results. In the second study, 26 rubella-susceptible children were inoculated and maintained in close association with 22 susceptible contacts. Again there was no clinical disease and no evidence of communicability.

This work has major scientific import for the following three reasons: (1) it provides the first evidence that rubella virus can be attenuated by laboratory manipulation; (2) it provides the first "marker" techniques that are capable of distinguishing between virulent and attenuated rubella viruses short of clinical trial, and (3) it provides an attenuated strain of the virus that is promising for further investigation in the search for a vaccine.

The Section on Viral Genetics has reported results of note in several basic research projects. In studying viral latency (Proj. No.52, p.131) it was found that the virogenicity of virogenic tumor cells (the ability of tumor cells containing subviral particles of simian virus 40 to infect normal cells) was dependent upon viability of the tumor cell as well as upon direct contact of these cells with the normal indicator cells.

In other investigations this Section again turned to the highly sensitive qualitative complement-fixation techniques which in the past year's annual report (Proj. No.91, p.133) had been shown capable of detecting subtle antigenic differences between closely related viruses. Their current work with the technique demonstrated that the Freund and Rauscher mouse leukemia viruses appear to be antigenically identical.

The Section on Immunology has developed two new procedures for detecting mumps virus antibodies. Both of these methods (a modified hemagglutination-inhibition test and a plaque-reduction neutralization test) are more sensitive than the conventionally employed antibody assay techniques. These new procedures were developed in anticipation of future needs in research and control obligations concerned with the prevention of mumps.

LABORATORY OF VIRAL IMMUNOLOGY

Control Program

July 1, 1965 through June 30, 1966

Antibody Assays: Using virus neutralization or hemagglutination-inhibition tests.

Immune Serum Globulin (Human)

Number of Lots received	239
Poliovirus Potency tests	227
Number of tissue cultures	57,204
Measles Potency tests (HAI)	295
Number of titrations	1,180
Potency tests (Neut.)	27
Number of tissue cultures	2,160

Poliomyelitis Immune Globulin (Human)

Number of lots received	18
Potency tests	18
Number of tissue cultures	4,536

Measles Immune Globulin (Human)

Number of lots received	5
Potency tests (Measles neut.)	20
Number of tissue cultures	1,600
Potency tests (Measles HAI)	10
Number of titrations	40

Testing of Reference Materials:

NIH Measles Reference Serum No. 1

Number of tests (Neut.)	22
Tissue cultures	890
Number of tests (HAI)	29
Number of titrations	116

NIH Reference Poliomyelitis Immune Globulin Lot 175

Measles neutralization tests	22
Number of tissue cultures	1,760
Measles HAI tests	29
Number of titrations	116

NIH Reference Poliomyelitis Immune Globulin Lots 174 and 175

Number of tests for Polio antibodies	46
Number of tissue cultures	11,592

Testing of Vaccines, Live, Attenuated:

Measles Virus

Number of lots received	183
Potency tests on lots	51
Number of tissue cultures	4,080
Number of tests on Reference Vaccines	86
Total number of tissue cultures	9,280

Poliovirus, Oral

Monovalent lots tested for d and t markers and virus content	2
Trivalent vaccines tested for virus content of all 3 types	10
Potency tests on vaccine samples from A.I.D.	3
Vaccine samples prepared and titrated for Laboratory of Pathology	49
Number of neurovirulence tests	40
Number of pre-inoculation monkey sera	1,225
Number of tissue cultures	14,700

Testing of Vaccines, Inactivated: (Using neutralization-hemagglutination or complement-fixation tests).

Measles Virus

Number of lots received	10
Potency tests on lots	4
Potency tests on Reference Vaccine	3
Guinea Pig sera tested (HAI)	300
Number of titrations	600

Mumps

Number of lots received	16
Potency tests on lots	3
Guinea Pig sera tested (CF)	80
Virus Titrations	13

Diphtheria, Tetanus, Pertussis and Poliomyelitis

Number of lots received	12
Monkey potency tests	3
Pre-inoculation sera	36
Post-inoculation sera	36
Number of tissue cultures	5,616
Chicken potency tests	11
Post-inoculation sera	220
Number of tissue cultures	25,440

Poliomyelitis

Number of lots received	17
Monkey potency tests	5
Pre-inoculation sera	60
Post-inoculation sera	60
Number of tissue cultures	9,360
Chicken potency tests	18
Post-inoculation sera	360
Number of tissue cultures	43,200
Number of monkey safety tests	6
Pre-inoculation sera	120
Number of tissue cultures	1,440

Poliomyelitis, Aluminum Phosphate adsorbed

Number of lots received	5
Chicken potency tests	5
Post-inoculation sera	100
Number of tissue cultures	9,600

Poliomyelitis Reference Vaccine (various pools)

Chicken potency tests	25
Post-inoculation sera	500
Number of tissue cultures	61,680

Poliomyelitis Reference Serum IIA3 and IIA4

Number of tests	26
Number of tissue cultures	6,500

Positive Chicken Serum Pool (Polio)

Number of tests	20
Number of tissue cultures	5,000

Control Sera (Chicken potency tests)	140
Number of tissue cultures	1,680

Serial No. DBS 49

1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Investigations concerning rubella virus and vaccine.

Previous Serial Number: DBS 49

Principal Investigator: Paul D. Parkman, M.D.

Other Investigators: Marvin S. Amstey, M.D.
Raymond Vaughn
Barbara B. Jackson
Eva Rau Brown
Harry M. Meyer, Jr., M.D.

Cooperating Units: Laboratory of Pathology, DBS
Laboratory of Virology and Rickettsiology, DBS

Man Years:

Total:	10.6
Professional:	5.1
Other:	5.5

Project Description: I. Studies of the biological characteristics of rubella virus.

In attempting to produce modification of the biological properties of rubella virus, the M-33 strain has been serially subcultured 100 times in GMK cells over a 2 to 4 year period. This protracted series of passages was initiated with virus originally recovered in GMK cells. Ordinarily infected cultures were incubated for one week before being tested for interference and subpassage. At the time of passage, cells and supernatant fluids from infected and control cultures were each inoculated into groups of fresh GMK cells. At intervals of 5 to 10 passages, 10 ml virus pools were prepared. Virus serially passaged less than 5 times was designated low passage virus (LPV); preparations passed 60 or more times were designated high passage virus (HPV). At intervals, virus preparations at various passage levels were tested for evidence of modification of biological characteristics.

Ordinarily, strains of rubella virus in throat washings or freshly recovered in GMK cell cultures do not produce marked cytopathic effect (CPE) in RK_{13} cell cultures, a fact which has limited the use of these cells for rubella virus isolation attempts. In evaluating virus strains passaged repeatedly in GMK cultures, it was apparent that this characteristic was modified; HPV strains produced rapid and complete RK_{13} cell destruction. A comparison of the time of appearance and rate of development of CPE in 4th and 77th passage levels of M-33 strain showed the low passage level virus required 14 days to manifest cytopathic changes; the high passage produced CPE in 7 days. Cytopathic effect progressed to complete destruction of the monolayers in six days with the HPV strain. LPV required twice as long

to develop the same cytopathic changes. Further experiments verified these differences. At GMK passage levels 2, 3, 4 and 9, the onset of CPE was delayed and the cytopathic endpoint titers were consistently 1.3 to 4.0 $\log_{10}$ units lower than the comparable interference titration value. At higher passage levels (22nd for M-33) this relationship was reversed so that the titer indicated by the cytopathic system was always equal to or higher than the interference titer. The time of appearance of CPE and its rate of progression in HPV preparations were similar to those observed with rubella viruses adapted to produce CPE in RK_{13} cultures by 15 to 20 serial passages in these cells.

Since RK_{13}-adapted rubella virus has been shown to produce plaques in RK_{13} cell cultures, several passage levels of virus strain M-33 were examined for possible differences in their ability to produce plaques in this cell line. In these experiments PFU titers were compared with titers obtained by the interference technique. Again, virus behaved differently at low and high passage levels. The M-33 strain in 5th to 23rd passage failed to produce plaques. Passage levels 63 and 74 of M-33 produced plaques indistinguishable from those observed with RK_{13} adapted viruses; from 1.2 to 1.7 $\log_{10}InD_{50}$ were required to produce one plaque forming unit in these experiments.

Studies of interferon production by several passage levels of rubella virus have been performed in collaboration with Mrs. Hope E. Hopps, LVR. Assays of culture fluids of primary kidney cell cultures infected with 4th and 74th passage rubella virus and rendered free of infectious virus have shown evidence of interferon production. In preliminary experiments GMK cells infected with either passage level appeared to produce interferon; the titers induced by the HPV appear to be 2 to 4-fold greater than that of LPV.

Earlier studies indicated that rhesus monkeys inoculated with rubella virus developed a characteristic pattern of infection. Using this experimental model, comparisons were made between infections resulting from inoculation of LPV and HPV strains. Animals were inoculated intravenously or intramuscularly. Pharyngeal, rectal swab specimens and blood were collected every 2 to 3 days for 3 weeks; sera for antibody determinations were obtained at 2 to 8 day intervals during the first month after inoculation and then at 2 to 4 week intervals for 3 to 4 months. In each experiment uninoculated monkeys (cage controls) were housed with those inoculated; other susceptible animals were kept in adjacent cages (room controls). Serial serum specimens were obtained from control animals for at least 8 weeks. Monkeys were examined for signs of illness when specimens were collected. None of the animals showed clinical signs of rubella. Results indicated that neutralizing antibody developed in all animals inoculated with LPV and in 93% of those given the HPV. Antibody was first detectable between the 9th and 20th days in both groups; titers obtained 4 weeks after inoculation ranged from 1:2 to $\geq$ 1:64/0.1 ml for animals given LPV and from 1:2 to 1:16 for those receiving HPV. Twelve of twenty four LPV-inoculated animals exhibited viremia prior to the appearance of detectable antibody. Virus excretion from the respiratory tract was demonstrable in 96% of these animals on one or more occasions between the 4th and 17th days

after inoculation and rectal excretion of virus was common. Transmission of infections occurred to 2 of 3 susceptible cage control monkeys and to 1 of 4 room controls. Animals inoculated with M-33 strain HPV showed a strikingly different pattern. Of the 14 animals inoculated with HPV, 9 received 5.5 to 6.3 $\log_{10}InD_{50}$ intravenously and 5 were given 3.5 $\log_{10}InD_{50}$ intramuscularly. These HPV-inoculated animals did not show viremia or virus excretion in respiratory or intestinal secretions and the experimental infections were not transmitted to any of 14 cage or 13 room controls.

Comparisons were made of infections produced in rhesus monkeys by LPV and HPV of M-33 strain rubella virus inoculated directly into the central nervous system (CNS). Each animal was injected with 0.5 ml bilaterally into the thalamus and 0.25 ml into the cisterna magna. Specimens for virus recovery and serologic study were collected every 2 to 7 days as described above. The LPV infections in 29 such inoculated rhesus monkeys have not been associated with detectable clinical illness or pathologic CNS changes. Antibody responses were observed in each animal by the 18th day. Virus was recovered from the CSF of 100% of those tested; from the pharynx of 60% and from the blood of 47%. In one instance, infection was transmitted to a control animal. In experiments with HPV, 19 animals were inoculated with 4.9 $\log_{10}InD_{50}$ of 77th passage level rubella virus. None of the monkeys showed clinical evidence of neurologic disease. Antibody developed in each of the inoculated monkeys but not in the controls. Virus was regularly recovered from all CSF specimens obtained 5 days after inoculation and in 14 of the 19 specimens collected on the 12th day. In contrast with the pattern observed after infection with LPV, viremia was detected in only two of the animals and only one shed virus from the respiratory or intestinal tract. In this monkey, virus was recovered from nasopharyngeal and rectal swab specimens on one occasion, 12 days after inoculation. Ten monkeys were sacrificed at 21 days, and the brains and spinal cords examined pathologically. Sections of central nervous system tissue including lumbar and cervical enlargements of the spinal cord, medulla, pons, midbrain, cerebellum and cerebral hemispheres failed to reveal any evidence of abnormality other than that resulting from the trauma of the inoculation procedure.

As reported previously, infection of control animals caged with LPV-inoculated monkeys occurred consistently. In several experiments, the incidence of contact infection varied between 13 and 67%. In contrast, HPV was not communicable to cage controls. In these experiments, 24 susceptible animals were each caged for 4 to 6 weeks with animals infected with 74th or 77th passage M-33 virus; none developed evidence of contact infection at any time during the 7 to 20 weeks of observation. Infection of susceptible monkeys caged separately in the same room with inoculated animals was infrequent with LPV and was not observed with HPV.

The antibody responses of animals inoculated with 74th or 77th passage level rubella virus resembled those observed following infection with 2nd to 20th passage preparations. Thus far, 52 of 53 susceptible monkeys inoculated parenterally with HPV developed neutralizing antibody. Tests on serial serum samples showed that antibody appeared 10 to 20 days after inoculation. Titers stabilized 1 to 2 months following infection. Presence of antibody induced

by previous rubella virus infection has been shown to protect monkeys against reinfection. A comparison of experiments in which monkeys originally infected with LPV or HPV were challenged intravenously with 4th passage level virus, showed that of 8 animals originally infected with LPV, 7 had antibody titers from 1:4 to 1:64 and showed no change in antibody level following challenge. The remaining monkey with detectable antibody at a 1:2 serum dilution developed a 16-fold increase in titer. Four simians immunized 4 to 5 months previously with 74th passage HPV were challenged intravenously with 6.1 $\log_{10}$InD$_{50}$ of 4th passage virus. Two, selected because of low antibody levels (1:2 and 1:4) showed 4 to 16-fold increments in titer. The remaining 2 animals, both with levels of 1:16, did not show significant increase in antibody titer. Rubella virus could not be recovered from nasopharyngeal, pharyngeal or rectal swab specimens or heparinized bloods collected on 9 separate occasions from each of the 4 animals between the 2nd and 21st day after inoculation, and none of 4 cage or 2 room control monkeys included in the experiment showed evidence of contact infection.

The passage levels at which the significant biological changes occur producing the modification of these experimental infections, have yet to be ascertained. When such information becomes available, it will be feasible to correlate the various in vitro "markers" (CPE in RK_{13} cells and interferon production) with modified in vivo infections. The modifications shown by HPV are all in the direction desired for the characteristics of a live, attenuated rubella virus vaccine. To prove the practical value of laboratory "markers" of attenuation however, requires human experimentation. Such studies have been carried out with 77th passage level rubella virus and are reported in the following section.

Project Description: II. Studies of rubella virus infections in man.

The laboratory data presented in Part I suggested that high passage level rubella virus might be suitable for a live rubella virus vaccine. Proof of this required clinical trial to document whether these laboratory changes were accompanied by attenuation and loss of communicability for man. A pool of live, attenuated rubella virus suitable for use in man was prepared in primary African green monkey kidney (GMK). This material represented the 77th GMK passage level of M-33 strain rubella virus and was designated HPV-77. It was prepared and safety tested in conformance with the U. S. Public Health Service Regulations pertaining to the manufacture of live, oral polio and live, attenuated measles virus vaccines (Biological Products, PHS Regulations, Title 42, part 73.110-73.118 [p. 25-31] and 73.140-73.146 [p. 4-40]). Results of these extensive tissue culture and animal safety tests have been reported in detail in the IND submitted by this laboratory. The clinical trials with this experimental vaccine have been performed at the Arkansas Children's Colony, a state-supported institution for the mentally retarded. This institution provides complete care for approximately 750 retarded children. These children were housed in small, 32-bed cottages, which function as independent units, under circumstances which allowed effective

isolation of study children with minimal interference in their normal activities. Essentially the entire staff of the Children's Colony were tested for rubella neutralizing antibody to insure that those personnel in contact with study children were immune. Informed consent was obtained from the parent or guardian of each child included in the project.

The study was carried out in two phases. The first phase involved 16 susceptible children living in a single cottage. Eight of these were inoculated with 3.9 $\log_{10}TCD_{50}$ of HPV-77, subcutaneously. The remaining 8 children acted as susceptible contact controls for detection of communicability of the HPV-77 strain. Specimens of heparinized blood were collected at 2 to 5 day intervals from the 6th to the 20th day from inoculated children and throat swabs were taken daily for 52 days from both inoculated and controls. Sera for antibody studies were obtained weekly from both groups. None of the children developed illnesses attributable to the vaccine; specifically no consistent pattern of fever, rash, significant lymphadenopathy or upper respiratory symptoms was demonstrable. Neutralizing antibody responses were detected in all of the 8 inoculated. Antibody first appeared in sera collected on the 20th day after inoculation; by the 28th post-inoculation day, all 8 had developed neutralizing antibody. Viremia was not observed in any of the children. Virus was recovered from throat swabs collected from 6 of the 8 children on two or more occasions during the period from 10 to 20 days following immunization. None of the 8 susceptible control children, living in close proximity developed antibody during 56 days of observation. Rubella virus was not recovered from their daily throat swab specimens, thus, despite shedding of the HPV-77 in the pharyngeal secretions, transmission of infection did not occur. The HPV-77 virus was shown to be attenuated and capable of stimulating the formation of rubella virus neutralizing antibodies.

On the basis of these encouraging results a second phase clinical trial was begun in a slightly larger group. The experimental design was similar; the study group was comprised of 48 susceptible children, divided among 3 cottages. Twenty-six received the same vaccine preparation subcutaneously. Twenty two children served as uninoculated controls. Specimens were collected as described for Phase I except that blood for viremia studies was obtained in a rotational sequence so that information relating to viremia would be available daily from day 7 to day 21. Thus far, the results of this study confirm those of Phase I. Neutralizing antibody developed by day 28 in 24 of 26 inoculated. Viremia did not occur; virus shedding in pharyngeal secretions was first detected on day 7. The last swab specimen yielding virus was collected on day 24. On any given day, the maximum number of throat specimens containing virus was 34% (days 12, 13 and 14). Although studies are incomplete at this point, none of the control children have shed virus or developed antibody. During the course of these studies an epidemic of natural rubella involving 9 clinically apparent cases occurred in a nearby cottage. Studies of specimens collected from these children are incomplete. Thus far, however, virus has been recovered from 100% of throat swabs taken 24 to 48 hours before the onset of rash, on the day of rash and on the first day after the eruption appeared. Viremia has been detected in two blood specimens collected prior to onset of rash.

Following the index case in this cottage, 8 of 10 susceptible individuals developed rubella. Preliminary observations suggest that at least 2 of the remaining seronegative persons developed antibody without evident clinical illnesses. These findings confirm earlier studies of the virologic events in rubella. They also provide a unique control on the efficacy of the rubella virus isolation procedures, and establish the high degree of communicability of natural rubella under conditions exactly comparable to those of the vaccine study children.

Project Description: III. Studies directed toward the standardization of serological tests for rubella.

Data accumulated over the past year has continued to confirm the utility of the neutralization tests performed in tube cultures of a continuous line of rabbit kidney cells (RK_{13}). This test has been shown to be of use in the screening of rubella susceptible and immune individuals. The major disadvantages of this test system are (1) the technical effort required in testing large numbers of sera and (2) the relatively long 3-week period of time required before final results are obtained.

Efforts have been directed toward this problem in four different areas: 1) use of a new cell line designated RK-T-17 for the standard neutralization tests; 2) metabolic-inhibition tests in RK_{13} and RK-T-17 cells; 3) rubella complement-fixation tests and 4) continuing studies with fluorescent antibody techniques.

1) The RK-T-17 cell line represents a high passage of cells originally derived from rabbit kidney. The cultures are composed of epithelial-like cells growing in confluent sheets. Ordinarily these cultures can be maintained in good morphological condition for two weeks. Rubella viruses produce definite cytopathic effect in these cells within 4-5 days and terminal virus dilutions ordinarily show CPE by the 10th day. An excellent correlation was found between titrations of RK_{13} adapted virus in RK_{13} cells (4.5 $\log_{10}TCD_{50}$) and RK-T-17 cells (4.4 $\log_{10}TCD_{50}$). Virus preparations passed 4 and 77 times in African green monkey kidney cell cultures also were found to produce CPE in these cells. They appeared to be less sensitive for primary isolation of rubella virus. Of four clinical specimens containing virus, only two produced CPE in two RK-T-17 passages. In a single test of the effect of incubation at 35°C, 32°C and 29°C, CPE appeared most quickly and the highest TCD_{50} titers were obtained in cultures held at 35°C. Rubella virus antibody was readily detected in neutralization tests performed in these cells. The geometric mean antibody titers of five determinations in RK-T-17 was 1:32/0.1 ml; the same figure for 17 tests of this human serum in RK_{13} was 1.31/0.1 ml. Each of 10 sera tested, determined to be antibody-free in RK_{13} cells, was also negative for rubella antibody when tested from 2 to 6 times in RK-T-17 cultures. The preliminary results suggest that these cells show promise for more rapid rubella neutralization tests.

2) Preliminary investigations have been carried out to establish the suitability of the RK_{13} cells and more recently, the RK-T-17 cell line for metabolic-inhibition tests. Following establishment of optimal cell count (100% cell survival over a 2-week period without refeeding) and optimal

virus concentration (100% inhibition of normal cell metabolism at challenge virus concentration), rubella sera of known antibody content were tested to accumulate data on the reproducibility of the test. The results of 14 tests indicated that the inhibition of RK_{13} cell metabolism was not rapid enough to permit the practical determination of endpoints. Variation in titers between different tests ranged between 4 and 8-fold. Recently the RK-T-17 line has been tested to establish optimal cell and virus concentrations. Further tests are planned to investigate the reproducibility of rubella antibody levels in tests using these cells.

3) A complement-fixation (CF) test utilizing a standard Bengston technique with commercial antigens was developed. A number of sera from children with clinical rubella were tested for CF antibody in acute and convalescent phase paired specimens. As reported previously the CF antibody apparently rises later and to a lower level than neutralizing antibody. Sera from adults without a history of recent rubella infection show little or no CF activity when tested at a 1:8 dilution.

4) In continuing studies of rubella immunofluorescence, an indirect technique has been developed using RK_{13} cells and anti-rabbit or anti-human gamma globulin fluorescein conjugate. RK_{13} cells infected with rubella virus have reliably shown a granular pattern of intracytoplasmic antigen after 4 days of incubation when stained by this procedure. The technique however, still does not appear sufficiently sensitive or reliable for routine detection of rubella antibody. The procedure may be useful in other studies currently in progress for detection of rubella virus in other types of culture and tissues.

Project Description: IV. Studies of the antigenicity of inactivated preparations of rubella virus.

No work in this phase during reporting period.

Project Description: V. Studies of possible rubella virus oncogenic effects.

Although to date no evidence has been produced suggesting an oncogenic role for rubella virus, experimental studies in suckling hamsters have been continued. Earlier experiments (DBS 49 Pt.V) failed to suggest that low passage level rubella virus produced oncogenic effects in hamsters. To determine the effect of serial passage in GMK cultures, 60 baby hamsters less than 24 hours of age were inoculated with the 77th passage rubella virus pool (HPV-77). These animals have been observed daily for evidence of illness and development of palpable tumors. No symptoms have developed in inoculated animals. None of the surviving hamsters have developed tumors during the 7 months since inoculation. There is no evidence to date that rubella virus induces tumors in this animal species.

Project Description: VI. Studies of rubella virus infection in experimental animals.

During the past year studies in experimental animals have been primarily directed toward elucidation of the behavior of low and high rubella virus passage levels (see above). Work is currently in progress on studies of high passage rubella virus infections in pregnant rhesus monkeys. These studies are being performed according to procedures which have been reported from this laboratory. Virologic data are not yet avaliable; however, no adverse effects have been observed on the course of the pregnancies or fetuses observed thus far.

Significance to Bio-medical Research and the Program of the Division:

All work pertains to problems concerned with the prevention of rubella and the control of the rubella vaccine.

Honors and Awards: Dr. Parkman--

Superior Service Award, Dept. of Health, Education, and Welfare, April, 1966.

Letter of Commendation from President of the United States, Lyndon B. Johnson, May 5, 1966.

Dr. Meyer--

Meritorious Service Medal of the Department of Health, Education, and Welfare, United States Public Health Service, 1966.

Letter of Commendation from President of the United States, Lyndon B. Johnson, May 5, 1966.

Publications:

Parkman, P. D., Phillips, P. E., Kirschstein, R. L. and Meyer, H. M., Jr. Experimental Rubella Virus Infection in the Rhesus Monkey, J. Immunol. 95: 743-752, 1965.

Parkman, P. D., Phillips, P. E. and Meyer, H. M., Jr.: Experimental Rubella Virus Infection in Pregnant Monkeys, Amer. J. Dis. Child. 110: 390-394, 1965.

Serial No. 46

1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Investigations Concerning Measles Virus and Vaccine

Previous Serial Number: DBS 46

Principal Investigator: Harry M. Meyer, Jr., M.D.

Other Investigators: George Stewart, M.D.
Robert D. Douglas
Eva Rau Brown

Cooperating Units: Laboratory of Pathology, DBS.

Man Years:

Total:	3.1
Professional:	1.0
Other:	2.1

Project Description: Study of virulent and attenuated measles virus in monkeys.

In the Laboratory virulent and attenuated measles viruses can be differentiated by the characteristics of the infections induced in inoculated measles-susceptible rhesus monkeys. However, even virulent strains do not produce histopathological changes when inoculated directly into the central nervous system (CNS).

Some have reported that African green monkeys were more susceptible to CNS inoculation. To evaluate this claim, we inoculated groups of measles-susceptible African green monkeys intracerebrally and intracisternally with either low passage (virulent) or high passage (attenuated) Edmonston strain measles virus.

The results were similar to those obtained in the earlier experiments with rhesus monkeys. No CNS histopathological changes were caused by either virus. However, virulent virus produced infections characterized by rash, viremia, virus shedding from respiratory secretions and the presnece of virus in cerebrospinal fluid. These features were absent in the infections produced by the attenuated virus.

Significance to Bio-medical Research and the Program of the Division: All work by the LVI with measles virus or measles vaccines is directly related to the resolution of problems pertaining to the control of measles.

Proposed Course of Project: Further studies will be made of the biological differences between virulent and attenuated measles viruses.

Honors and Awards: None.

Publications: None.

Serial No. 48

1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Investigation Concerning Mumps Virus and Vaccine

Previous Serial Number: DBS 48.

Principal Investigator: Harry M. Meyer, Jr., M.D.

Other Investigators: George Stewart, M.D.
Robert D. Douglas
Eva Rau Brown

Cooperating Units: None

Man Years:

Total:	2.93
Professional:	.83
Other:	2.1

Project Description: Part I. Studies of the biological characteristics of mumps virus. Originally the biological characteristics of the Enders high egg passage strain of mumps virus were compared with those of a low passage (Sporn strain) mumps virus (1964-65 annual report).

This line of work has been expanded to include more detailed comparisons of the Sporn strain and another low passage (Saunder's strain) mumps virus isolated in this laboratory. These studies have concentrated on in vitro behavior of the viruses in a number of primary and continuous live cell cultures and on in vivo properties of the strains as shown by inoculation of monkeys.

In preliminary experiments both rhesus and African green monkeys were inoculated with either crude virus (from the patient's saliva specimens) or with tissue culture propagated virus. There was no evidence of clinical disease in the animals although infection occurred as demonstrated by the development of complement-fixing, hemagglutination-inhibiting and neutralizing antibodies for mumps virus. Viremia and virus shedding in the saliva and respiratory secretions could not be detected.

Project Description: Part II. Studies directed toward the standardization of serologic tests for mumps.

Considerable progress has been made in this area during the past year. Two new serologic procedures have been developed and extensively

studied in comparison with the conventional serologic tests for mumps.

A modified hemagglutination-inhibition (HAI) test using a Tween-80-ether treated antigen has been shown to give 2 to 4-fold higher mumps antibody titers than the standard HAI technique. A plaque reduction neutralization test employing the MA-104 line of continuous rhesus monkey kidney cells proved to be 2 to 10 times more sensitive than the routine neutralization method (endpoint readings by cytopathic effect). These two new methods have a number of potential uses in our mumps reasearch and biologics control program.

Significance to Bio-medical Research and the Program of the Division: All of this work directly pertains to research problems in the prevention of mumps and control of mumps vaccines.

Honors and Awards: None.

Publications: None.

Serial No. 47

1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Investigations Concerning the Smallpox Group of Viruses and Smallpox Vaccine.

Previous Serial Number: DBS 47

Principal Investigator: Harry M. Meyer, Jr., M.D.

Other Investigators: George Stewart, M.D.
Robert Douglas
Eva Rau Brown

Cooperating Units: None

Man Years:

Total:	2.93
Professional:	.83
Other:	2.1

Project Description: Study of immunity to vaccinia virus in experimental animals.

Rhesus monkeys have been used to study the cutaneous and immunologic response to inoculation with vaccinia virus. Our interest has been focused on determining the importance of the route of inoculation and virus dose on immunity.

Animals have been vaccinated by (1) the multiple pressure technique, (2) jet inoculation, (3) intradermal inoculation and (4) subcutaneous injection. Inoculae have contained from 10^2 to 10^8 $TCID_{50}$ of vaccinia virus.

By varying the virus dose and route of inoculation, it was possible to evoke antibody production without eliciting a dermal response. This simulated the type of human response observed during our clinical investigations with dilutions of smallpox vaccine given by jet injection.

These monkeys are being challenged with a percutaneous inoculation of smallpox vaccine to determine the degree of immunity conferred by the primary, atypical vaccinia infections.

Significance to Bio-medical Research and the Program of the Division: All of this work directly pertains to research problems in the prevention of smallpox and control of smallpox vaccines.

Proposed Course of Project: These investigations are being continued along the lines described.

Honors and Awards: None.

Publications: None.

Serial No. DBS 52

1. Laboratory of Viral Immunology
2. Section on Test Development
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on Simian Virus 40.

Previous Serial Number: DBS 52

Principal Investigator: Paul Gerber, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	0.5
Other:	1.0

Project Description: Part I. Transfer of subviral infectivity from SV_{40}-induced tumor cells to indicator cells.

Objectives: Study of the phenomenon of viral latency.

Methods Employed: Conventional virologic techniques.

Major Findings: Demonstration of virogenicity requires viability as well as direct contact of the tumor cells with the indicator cells. The number of detectable virogenic tumor cells depends on their plating efficiency. The tumor cells retain their virogenicity following prolonged passage in the presence of 10 ug/ml of Cytosine Arabinoside.

Significance to Bio-Medical Research and the Program of the Division: Study contributes basic information to our knowledge of virus-cell relationships.

Proposed Course of Project: This project is continuing.

Project Description: Part II. Search for antibodies to SV_{40} tumor antigen in sera of children immunized with poliomyelitis vaccines containing infections SV_{40}.

Objectives: Detection of anti-SV_{40} tumor antibodies in humans.

Methods Employed: Sequential sera of children following their last booster dose of poliomyelitis vaccines, were tested by complement-fixation and/or by indirect fluorescent antibody techniques.

Major findings: No detectable anti-SV_{40} tumor antibodies were present in the sera of 30 children who were immunized with poliomyelitis vaccines containing infectious SV_{40}.

Significance to Bio-medical Research and the Program of the Division: This is an integral part of the DBS control and testing program.

Proposed Course of Project: No further work is planned at this time.

Honors and Awards: None.

Publications:

Gerber, P. Induction of virus synthesis in virogenic hamster tumor cells. Year Book of Cancer, Vol. X, 1965.

Gerber, P. Studies on the transfer of subviral infectivity in SV_{40}-induced tumor cells to indicator cells. Virology 154: no. 3634, p. 833, 1965.

Serial No. DBS 91

1. Laboratory of Viral Immunology
2. Section on Test Development
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Immunological Studies of Murine Leukemia Viruses.

Previous Serial Number: None

Principal Investigator: Paul Gerber, Ph.D.

Other Investigators: Timothy O'Connor, Ph.D., NCI

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	0.2
Other:	1.0

Project Description: Antigenic analysis of Rauscher and Freund leukemia viruses.

Objectives: Determine the antigenic relationship between two murine leukemia viruses.

Methods Employed: Viruses were derived from the plasma of viremic Balb/c mice. Purification and concentrations were achieved by differential centrifugation and by density gradient techniques. Immune sera were prepared by vaccination of Balb/c mice with formaline-inactivated spleen extracts of leukemic Balb/c mice. A quantitative complement fixation test was used to study the antigenic character of these viruses.

Major Findings: 1) Both viruses formed a single band in a sucrose gradient at a density of 1.16. This material contained a high concentration of leukemia virus particles as determined by electron-microscopic examination and was in fixed complement. 2) The complement-fixation curves observed in the cross-reactions indicated close antigenic relationships between Freund and Rauscher leukemia virus. 3) Highly purified virus preparations following repeated density gradient centrifugation contained detectable quantities of host antigen.

Significance to Bio-medical Research and the Program of the Division: This study contributes basic information to our knowledge of the properties of murine leukemia viruses.

Proposed Course of Project: The results of this study will be described in a paper for publication.

Honors and Awards: None.

Publications: None.

Serial No. DBS 90

1. Laboratory of Viral Immunology
2. Section on Test Development
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on Rabies Virus.

Previous Serial Number: None

Principal Investigator: Paul Gerber, Ph.D.

Other Investigators: None

Cooperating Units: Communicable Disease Center, Rabies Laboratory.

Man Years:

Total:	1.2
Professional:	0.2
Other:	1.0

Project Description: Development of the in vitro methods for antibody for determination of immune response following vaccination with Rabies Vaccines.

Objectives: Devise a sensitive in vitro serological test for detection of rabies antibodies.

Methods Employed: Immunological techniques adaptable to viral antigens.

Major Findings: This project was started just three months ago. No definite results are available at this time.

Significance to Bio-medical Research and the Program of the Division: A sensitive in vitro antibody test for Rabies would be of great value to determine the immune response to vaccination.

Proposed Course of Project: Project will continue.

Honors and Awards: None.

Publications: None.

Serial No. 50 and 51
1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Investigations Concerning Polioviruses and Poliomyelitis Vaccines.

Previous Serial Number: DBS 50 and 51.

Principal Investigator: Marvin S. Amstey, M.D.
Paul D. Parkman, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.25
Other:	0.25

Project Description: Studies on the use of dimethylsulfoxide and other agents to enhance poliovirus RNA infectivity.

Dimethylsulfoxide (DMSO) was tested for its ability to enhance polio-RNA infection because of its unique ability to enhance absorption of a wide variety of compounds. DMSO enhanced polio-RNA infection over 1000-fold. The maximum RNA infectivity was achieved with 40% DMSO at room temperature with an absorption period of 20 minutes. DMSO was found to enhance polio-RNA infection to a greater degree than did protamine, histone, DEAE-dextran or hypertonic $MgSO_4$.

Significance to Bio-medical Research and the Program of the Division: This work pertains to increasing our knowledge of polioviruses and the control of poliomyelitis.

Proposed Course of Project: These results are to be summarized for publication.

Honors and Awards: None.

Publications: None.

Serial No. 53
1. Laboratory of Viral Immunology
2. Section on Serology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: The application of tissue culture methods to inherited metabolic disorders.

Previous Serial Number: DBS 53.

Principal Investigator: B. W. Uhlendorf, Ph.D.

Other Investigators: D. S. Fredrickson, M.D., NHI
M. B. Mock, M. D., NHI
J. N. Kanfer, Ph.D., NINDB
S. H. Mudd, M.D., NIMH

Cooperating Units: Laboratory of Metabolism, NHI
Laboratory of Neurochemistry, NINDB
Laboratory of General and Comparative Biochemistry, NIMH

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description: Objectives.

A. Niemann-Pick Disease.

1) To be able to diagnose and/or confirm diagnosis of NPD using cell cultures grown from skin or bone marrow biopsies or from amnion cultures of suspected cases. (At the present time, definitive diagnosis depends on analysis of surgically obtained liver biopsy.)

2) To establish whether cell cultures can be used to detect the heterozygous carrier of NPD. (No test for this is available and one would be useful for genetic counseling.)

3) To learn more about the biochemistry of NPD by experiments using cell cultures.

B. Homocystinuria.

1) To determine whether cell cultures will provide a source of the enzyme cystathionine synthase which is mutant or missing in homocystinuria.

2) To complete a study of transsulfuration in diploid and heteroploid or "transformed" cell cultures.

Methods Employed: Standard methods of cell culture, chemical analyses and enzyme assays were used.

Major Findings: A. Niemann-Pick Disease.

We have demonstrated that a defect, expressed as increased levels of sphingomyelin (and cholesterol) persists in long term cell cultures derived from tissues of NPD patients. Preliminary tests show the absence in these cultures, of the recently discovered enzyme sphingomyelinase. These results suggest that definitive diagnosis can be made using cell cultures. The possibility that cell cultures from heterozygotes contain an intermediate level of sphingomyelinase is under investigation. For this purpose we are growing in serial passage cell cultures from skin biopsies of parents of six confirmed or suspected NPD patients. A chemical characterization of the sphingomyelins produced by NPD and control cultures is progressing as well in a study of the extent of the cholesterol involvement in these cultures.

B. Homocystinuria.

Preliminary results indicate that whereas tissue such as skin and kidney do not exhibit cystathionine synthase activity, cell cultures derived from these tissues do, and that the activity may be further increased when the cells become aneuploid. Also under study are two other enzymes involved in cystine synthesis. We are using diploid and SV_{40}-"transformed" human cells, established lines of heteroploid human cells and diploid and heteroploid cells of non-human origin. These enzyme activities are interesting in relation to gene expression and control mechanisms and in relation to known differences in nutritional requirements of human diploid and heteroploid cells for cystine.

Significance to Bio-medical Research and the Program of the Division:

Tissue culture is proving to be an important tool in the study of inborn errors of metabolism. The results of these studies may also be significant in the possible development of human diploid cell strains for vaccine production.

Proposed Course of Project:

This project is continuing.

Honors and Awards: None.

Publications:

Uhlendorf, B. W., Holtz, A. I., Mock, M. B. and Fredrickson, D. S.: Persistence of a metabolic defect in tissue cultures derived from patients with Niemann-Pick disease in "Inborn Disorders of Sphingolipid Metabolism", S. M. Aronson and B. W. Volk, Ed., Pergamon Press. (in press).

DCRT

DRFR

LVR

CONTRACTS

SUBJECT INDEX

INVESTIGATORS

PUBLICATIONS

Annual Report of the
Laboratory of Virology and Rickettsiology
Division of Biologics Standards
July 1, 1965 through June 30, 1966

During the past year we took a big stride toward the accomplishment of our goal — creation of a new and different kind of virological laboratory at DBS, not based on historical precedent or even recent needs, but taking into account the likely long range responsibilities of the Division. This approach, at first slow and relatively unproductive, gradually provides the required tools for the people who are trained to use them effectively. We have taken two years to build up the facilities, to introduce important current techniques, to establish coordinated laboratory programs and to reorganize the administrative set up.

By now only two of the five sections in the Laboratory remain more or less the same. Because of the continuing importance of respirovirus vaccines, the Section on Respiratory Viruses has been allowed to function as before, at least for the time being. Because the so-called Virus Biology Section has been concerned exclusively with antimicrobial and antitumor substances from mollusks, fundamental biological questions related to intracellular microbial parasitism have been increasingly often addressed to the former rickettsial group, now developed into Section on Intracellular Infection. The Tissue Culture Section has been pushed into establishing a research program in cell biology, consonant with the mission of providing the Division with good cell cultures, but no longer limited to matters of routine production. The other new Section (Experimental Virology) is rapidly developing a central immunological and serological facility for LVR, a laboratory capability for working with tumor viruses and a comprehensive research program in arthropod-borne viruses to insure for the NIH at large, and not only the Division, continuity of an intramural expertise in this specialized and complex field.

Thus, the Laboratory has taken on a new look - from immunofluorescence to electron microscopy, from DNA homology to tumor transplantation — the look of a laboratory with basic virological orientation, proper for a new laboratory in the new climate of contemporary biologics. The solid laboratory base will be most useful in designing future human volunteer and field population studies.

HIGHLIGHTS OF INDIVIDUAL PROJECT REPORTS

During the year the newly established arbovirus collection was greatly expanded to include other viral (and mycoplasma) antigens and antibody reagents (Proj. No. 75 p 186). This was a result of the decision to establish an immunoserology unit to serve as a nucleus for a major service and research component of LVR. At the time of this writing (April), 184 agents are in the working stock of 300 frozen lots of "master", "working", and "vaccine" virus pools, 112 antigens for complement fixation and hemagglutination-inhibition, and 122 antibody reagents (hyper-immune sera and ascitic fluids). Very recently 65 new arbovirus prototypes were received from Yale Arbovirus Unit (and not yet entered in the table); master and working pools will be produced shortly. Techniques of immunodiffusion and agar gel diffusion have been added to the already routinely performed quantitative complement fixation, hemagglutination-inhibition and immunofluorescence techniques.

A new study on the electronmicroscopic morphology of certain viruses was undertaken in collaboration with Dr. K. O. Smith, LBB. An early result was the determination of the myxovirus nature of the Kemerovo virus prototype (isolated by Soviet workers from ticks and humans). The agent was definitively identified as Newcastle disease virus by J. Casals and ourselves. Human epidemics due to Kemerovo virus have presented a serious public health problem in Siberia. Prof. Chumakov has been notified of our findings.

Owing to the Division's increasing responsibilities related to avian leukosis viruses, a major build-up of the required facilities has been accomplished during the year (Rep. Nos. 72 and 73 ,p.177 & 180). The combination of RIF tests in tissue culture, serological COFAL tests, and immunofluorescence covers the gamut of techniques necessary to cope not only with the potential RIF contamination of egg-produced vaccines, but prepares us for the future should the virus of Marek's disease of fowl prove to be a tumor virus potentially hazardous to humans receiving egg-produced vaccines (a reference collection of candidate Marek's virus agents is being set up). Such experience will serve as a base for other oncogenic virus work when the Division is called upon to work with candidate viruses for human cancers.

The control and research activities related to avian leukosis have become a major pre-occupation of several of our senior staff. Vaccines and other suspect materials were examined as required, by RIF test and also by complement fixation and immunofluorescence; four Rous sarcoma virus tumors (Bryan, Zilber-Carr, Prague & Armstrong) have been maintained in hamsters by transplantation; recently the tumor cells of all four were successfully adapted to tissue culture opening up a new area for research and development. A new and important method for determining fowl maternal RIF antibody (or virus) in egg yolk by immunofluorescence has been worked out. This should allow ready monitoring of chicken flocks and preparation of RIF-free cell cultures from chick embryos taken from eggs with yolk known to be negative by this specific immunofluorescence test.

To our knowledge, LVR is the only laboratory in the world today which possesses the required competency, facilities, and interest in arboviruses and avian leukosis viruses. This has prompted us to propose, in collaboration with other DBS laboratories, a comprehensive re-evaluation of Yellow Fever vaccine, hopefully leading to a safe and potent RIF-free yellow fever virus seed.

Current studies on adenoviruses serve to accumulate information which will be of help in re-establishing adenovirus vaccines to the armamentarium of respirovirus vaccines for use in man, and, in addition, will be of assistance in understanding the biology of adenovirus infection (Rep. No. 98 ,p. 156). During the past year, the early findings of Huebner and associates on the "hybridization" of adenovirus type 7 and SV40 were extended by showing that SV40 genetic material is apparently also enclosed within adenovirus type 3 capsids. Related to this are the current studies on the occurrence of SV40 antibody in the sera of people vaccinated with the adenovirus lots known to

contain SV40 genetic material and on the possible serological response to the "adeno-associated virus" particles known to be present in many adenovirus vaccines.

The Section on Respiratory Viruses continues to develop information which should be of assistance in improving the effectiveness of influenza virus vaccines. For instance, evidence has accumulated to indicate a marked recent shift in the antigenic composition of some of the contemporary strains of influenza B. This affects the Division's plans for the construction of the next vaccine formula.

Important data on the relationship of serum neutralizing influenza antibody levels and resistance to subsequent influenza virus challenge were obtained from a human volunteer study. Even more important information on the dynamics of continual change in the antigenic composition of influenza viruses may be obtained from a field study of immunologically virginal populations of several Pacific atolls. Some of the island groups experienced a severe epidemic of influenza in 1964; their antibody responses to epidemic infection are being followed and the response to monovalent vaccine will be examined. An additional group of nearly 1,000 people, without influenza A or B antibody, who had escaped infection during the 1964 epidemic, offers a unique opportunity to evaluate immunological responses to monovalent influenza virus vaccines.

For several years the Section has been concerned with the improvement in assay procedures to measure the potency of influenza virus vaccines. Experiments are underway to determine in vaccinated mice the degree of resistance to direct intranasal virus challenge and to relate the findings to those obtained in mice vaccinated in the conventional assay procedure, and to observations in volunteers selected to participate in influenza virus vaccine assay test.

Another family of viruses potentially capable of producing in man an illness which clinically might be mistaken for influenza was recently added by defining the relationship between African epidemic icterus virus isolated from humans and the murine hepatoencephalitis virus group (Rep. No. 60 p. 149).

As stated in the last annual report, the original rickettsial group of LVR was to extend its activities into the broad realm of mechanisms and dynamics of intracellular microbial parasitism, consonant with the proposed re-designation as Section on Intracellular Infection. Their studies have concerned interferon production, parasite-host-cell relationships, comparative electronmicroscopic morphology, and genetic relatedness of rickettsiae, mycoplasmas, and viruses. Interferon production does not seem to be a prominent feature of rickettsial proliferation in vivo. On the other hand, the level of interferon production may serve as a reliable marker for rubella virus attenuation. Careful collaborative electronmicroscopic studies of mycoplasmas, larger viruses and rickettsiae showed that the 3 groups of organisms are morphologically distinguishable from one another (Rep. No. 65 ,p. 165).

Studies on the characteristics of virulent and avirulent rickettsiae (typhus, spotted fever and scrub typhus) are continuing because of the need for a better understanding of the phenomenon of attenuation in relation to live rickettsial vaccines (Rep. No. 66 ,p. 168). Certain fractions of the killed typhus rickettsiae (soluble antigen, cell wall and intracellular protoplasm) are being compared with the intact organism to define their relative immunogenicity as a step toward more potent, purified vaccine (Rep. No. 59 ,p. 160). As a refinement, rickettsiae grown in cell culture rather than eggs, are now being tried. Initial steps toward developing a vaccine potency test using monkeys, rather than guinea pigs, have been taken by determining the susceptibility of rhesus and cercopithecus monkeys to epidemic typhus rickettsiae. In an effort to establish a back-up cell line for BS-C-1, particularly as needed for measles vaccine testing, a second line of cercopithecus kidney cells has been under test - this is designated BS-C-2 (MA-134) cell line. Extensive comparative studies indicate that BS-C-2 can satisfactorily replace the standard BS-C-1 line (Rep. No. 63 ,p. 163).

The Laboratory now possesses a competency to perform studies on the genetic relatedness of rickettsiae using the DNA homology techniques. The initial studies employed the DNA from R. quintana; the technique will be applied to other rickettsiae, mycoplasmas and probably viruses (Rep. No. 82 , p. 170).

The Tissue Culture Section continues to supply the entire Division with large numbers of primary and continuous cell cultures from verifiable sources and of high quality. Some 400,000 cell cultures in tubes and 150,000 in bottles of different sizes were issued during 1965. This service activity occupied practically the entire time of the Section's personnel but the new emphasis on research and development has resulted in the introduction of several new methods into production; these have improved the quality and quantity of cell cultures at sizable savings (Rep. No. 79 ,p.174). New research projects deal with studies on malignant cell transformation in vitro, using human diploid cells (WI-38 cells), which are of particular concern to DBS, having been proposed for use in human vaccines (Rep. No. 67 ,p. 172). This possibility has caused another project the purpose of which is to determine the presence of blood group or tissue transplantation antigen in cultured WI-38 cells, because of a potential hazard of sensitization of vaccine recipients (Rep. No. 83 ,p. 176).

Studies of the antitumor effects of clam extracts, presumably containing the active principle (paolins), have been extended to include adenovirus 12 tumors (virus-induced or transplanted) in hamsters, mouse leukemia (L-1210), mouse sarcoma 180, and mouse melanoma (S-91). Preventive treatment of newborn hamsters soon after inoculation with oncogenic adenovirus 12 seemed to inhibit tumor formation. Weaned hamsters with transplanted solid adenovirus 12 tumors shed the tumors and survived indefinitely when the clam extract was injected into and around each tumor. Mice with otherwise lethal melanomas, leukemias and sarcomas responded to clam extract treatment, sometimes in a most dramatic fashion. To allow general confirmation and extension of these challenging results, a large quantity of clam extract has been prepared by a

commercial laboratory on contract with the Division. The material will be used to supply the demands of other investigators at NIH and elsewhere and for further attempts at characterization, isolation, and identification of the active principle in clam extract. (Rep. No.74,p.182).

Hepatitis. The A-1 agent described some years ago as a virus recovered from an icterogenic human plasma pool, has now been definitely identified as a strain of Mycoplasma gallisepticum. Serological tests, by plaque reduction and growth inhibition techniques, of sera from various series of human cases of hepatitis, indicate some relation of this Mycoplasma to the disease. However, it has not been possible to re-isolate the Mycoplasma from the icterogenic pool. Also, more work is necessary to rule out anamnestic responses in the human cases tested and to be sure that significance can be attached to small rises in titer. The new approach to the A-1 agent suggests a large number of additional studies.

CONTRACT ACTIVITIES

A new contract (under No. PH-43-66-35) was made with Melpar, Inc. to prepare a large quantity of clam extract so that it can be made available for study at LVR and elsewhere. Some 250 gallons of shucked fresh clams (Mercenaria mercenaria) were purchased, frozen and are being gradually processed.

The initial interest in antiviral effects of the extract has gradually shifted to a concern with its antitumor properties. Accordingly, the techniques of extract preparation and of its assay for specific activity have been modified (see Dr. Li's Individual Project Report No. 74, p.182). Limited attempts at purification and concentration were made at Melpar by such means as Sephadex fractionation and high speed centrifugation followed by DEAE treatment.

SERVICE AND CONTROL FUNCTIONS

Most of the working hours in the Tissue Culture Section are spent on the purely service function of providing the entire Division with cell cultures of research quality from verifiable sources. In addition, some time is spent on a control activity of safety testing the inactivated virus vaccines in cell culture.

During the calendar year 1965 the following cultures were prepared by the Section - 398,150 tubes, 129,532 2-ounce bottles, 2,102 milk dilution bottles and 4,988 32-ounce bottles of both primary and serial cell cultures. Sizable quantities of rhesus, cercopithecus, and BS-C-1 cell cultures were used in the safety testing of live poliovirus and live measles vaccines. Large quantities of cercopithecus, RK-13 and MA-104 cell cultures were used in the rubella program. More than 27,000 ml of cell suspension were prepared from 9 serial cell lines to investigate the activity of clam extracts in vitro. Several lots of primary cells of rat, dog and guinea pig kidneys, mouse

embryo, monkey heart, monkey testes and monkey ovaries were processed on request from interested investigators. Production records of primary and serial cell cultures are summarized in Tables 1, 2 and 3.

The Respiratory Virus Section is responsible for potency testing of influenza and adenovirus vaccines. One hundred and eleven influenza virus vaccine lots were received in the laboratory of which 99 were manufactured for civilian use and 12 - for the military. Seventy-nine of the civilian vaccine lots were examined on the basis of the manufacturers' protocols only, 17 lots were selected for testing by DBS, and 3 lots were withdrawn by the manufacturer. Of the 12 military vaccines, 2 were withdrawn by the manufacturer and the other 10 were tested in our laboratory; the manufacturers' protocols were examined in all cases. (No adenovirus vaccines were submitted for release during this year).

The Avian Leukosis Unit of the Section on Experimental Virology performed 30 RIF tests on 23 live measles vaccines, all of which were found to be negative for the RIF virus by this procedure.

No rickettsial vaccines were submitted for testing by the Laboratory during this year.

Table 1
Report for Calendar Year 1965
CELL YIELD FROM PROCESSED ANIMAL TISSUES

Type of Tissue	Grams of Tissue Processed	Cells per Gram of Tissue
Kidney		
Monkey, rhesus	1,914	150×10^6
Monkey, cercopithecus	1,910	141×10^6
Rabbit	656	49×10^6
Embryo		
Chick	1,124	94×10^6
Mouse	32	117×10^6

Table 2
Report for Calendar Year 1965
NUMBER OF PRIMARY CELL CULTURES ISSUED

Type of Culture	Tubes	Bottles		
Kidney		2 oz.	MDB*	32 oz.
Monkey, rhesus	114,825	53,985	-	417
Monkey, cercopithecus	137,044	8,926	1,139	1,087
Rabbit	34,015	11,035	464	462
Embryo				
Chick	1,897	33,500	149	63
Mouse	-	-	-	32

*MDB - Milk dilution bottles

Table 3
Report for Calendar Year 1965
Number of serial cell cultures prepared for issue in tubes and bottles from 4,988 bottles (32 oz) maintained as cell stock

Type of Culture	Tubes	Bottles		
		2 oz.	MDB[a/]	32 oz.
Human				
HEp -2 (PD & Co)	1,615	265	11	1,478
HeLa (S-3 Eagle)	990	84	44	480
Human amnion (Lewis)	100	-	2	3
KB (Eagle)	130	-	3	136
Chang's Liver (Chang)	116	765	7	6
Animal				
Monkey heart (Salk)	60	-	-	-
Monkey, stable (Tytell)	370	-	-	-
Lilly monkey kidney (Hull)	285	650	4	114
BS-C-1 (Hopps)	52,996	4,182	215	42
MA-104 (MBA)	8,536	12,388	52	20
Rabbit kidney-13 (Beale)	44,701	3,460	12	582
NCTC 2472 (ATCC)	-	190	-	-
CCRF S-180 II (ATCC)	-	-	-	4
BHK-21 (Macpherson & Stoker)	120	100	-	-

a/ MDB - Milk dilution bottles

Serial No. 57
1. Virology & Rickettsiology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Office of the Chief

Previous Serial No: Same

Principal Investigator: Alexis Shelokov, M.D.

Other Investigators: None

Cooperating Units: None

Man Years
Total: 4.1
Professional: 0.6
Other: 3.5

Project Description:

Objective: Administration, planning and coordination of research and control activities of the Laboratory's sections.

The project furnishes supervisory and administrative services to all research projects and biologics control activities in the laboratory as follows: (a) Over-all planning of intramural research, collaborative (contract) and control functions and their coordination among and within the several Sections of the Laboratory, (b) supervision over personnel, maintenance, travel and correspondence, (c) requisitioning and supervision of equipment and supplies, (d) reviewing, editing and revising scientific and technical reports and manuscripts, (e) preparation of administrative reports, exhibits and other publication materials, (f) participating with other Laboratory Chiefs in staff functions for Office of the Director, including staff committees which ensure smooth operation of the Division in its many types of activity, (g) official liaison and communications with the manufacturers of viral vaccines and other biologics, (h) consultation services to individuals, academic institutions and government agencies, including liaison activities with other branches of the Service, foreign governments and international agencies, (i) maintenance of reference library and reprint files.

Honors:

1. Invited Lecturer - North Carolina Academy of General Practice, Durham, North Carolina, November 1-2, 1965 (Respiratory virus vaccines).

2. Invited Lecturer - U. S. Army Biological Laboratory, Fort Detrick, Frederick, Maryland, January 20, 1966 (Soviet hemorrhagic fevers).

3. Invited Lecturer - Washington School of Psychiatry, Washington, D. C. February 9, 1966 (Medicine in the Soviet Union).

4. Invited Lecturer - Wayne State University, Detroit, Michigan, May 18, 1966 (Epidemic Hemorrhagic Fevers: their epidemiology, etiology and clinical features).

5. Member, Scientific Advisory Board, Serum Reference Bank, World Health Organization, Yale University (appointed 1964-67).

6. Member, Scientific Advisory Board, Gorgas Memorial Institute, Washington, D. C. and Gorgas Memorial Laboratory, Panama (re-appointed 1965-66).

7. Member, Executive Committee, American Committee for Arthropod-borne Viruses (re-elected 1966).

8. Member, Committee for Arbovirus, National Institute of Allergy and Infectious Diseases (re-appointed 1966).

9. Consultant in Research (Virology) on the Graduate Council, The George Washington University, Washington, D. C. (appointed 1966).

Serial No. 60
1. Virology & Rickettsiology
2. Respiratory Viruses
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies concerned with influenza viruses

Previous Serial Number: 60 (Title changed)

Principal Investigator: J. A. Morris, Ph.D.

Other Investigators:
Marilyn Bozeman, M.S.(WRAIR)
Fred Davenport, M.D.(Univ. Michigan)
Jose David-Ferreira, M.D., Ph.D.(Portugal)
Andre Ferrari(New York)
Hideo Fukumi, M.D.(Tokyo)
Carleton Gajdusek, M.D.(NINDB)
Julius Kasel, Ph.D.(NIAID)
Vernon Knight, M.D.(NIAID)
Joseph Metzger, M.D.(Japan)
John Parker, Ph.D.(Micro. Associates Inc.)
Joseph Quilligan, M.D.(Loma Linda Univ.)
Roslyn Q. Robinson, Ph.D.(CDC Atlanta)
Mehmet Saglam, M.D.(Ankara, Turkey)
Merrill Snyder, Ph.D.(Univ. Md.)

Cooperating Units:
Walter Reed Army Medical Center
University of Michigan
Auto-Technicon Company
Kokuritsu Yobo Eisei Kenkyujo (Tokyo)
Gulbenkian Foundation (Lisbon)
Laboratory of Child Growth & Development, NINDB
Laboratory of Clinical Investigation, NIAID
406 Medical General Laboratory (Tokyo)
Microbiological Associates, Inc.
Loma Linda University
Communicable Disease Center, Atlanta, Ga.
Medical Command, Turkish Army (Ankara)
University of Maryland

Man Years:
Total: 7.2
Professional: 0.7
Other: 6.5

Project Description:

Objectives: Work on this project was directed toward collection of information which would be of assistance in improving effectiveness of influenza virus vaccines.

I. Studies concerned with antigenic structure of current epidemic influenza virus strains.

Outbreaks of influenza occurred in the latter part of 1965 on the east and west coasts of the United States. Outbreaks on the west coast were explosive and were found to be caused by influenza type A2 viruses which were indistinguishable antigenically from A2/Taiwan/1/64 (A2/Taiwan/1/64 is the influenza virus type A2 strain which was selected in 1965 for incorporation into the influenza virus vaccine for use in the United States during the 1965-1966 influenza season). Outbreaks on the east coast were moderate and were found to be caused by influenza type B viruses which fell into 2 markedly different sub-types. The influenza B viruses associated with respiratory disease outbreaks in the southeastern coastal states were similar antigenically to the prototype strain, B/Maryland/1/59 (B/Maryland/1/59 is the influenza virus B component in influenza virus vaccine produced for use in this country in 1965-1966); influenza B viruses associated with respiratory disease outbreaks in New England and in the District of Columbia were strikingly different antigenically from the prototype strain B/Maryland/1/59. This marked shift in the antigenic composition of some of the contemporary influenza B strains away from the prototype B/Maryland/1/59 will serve as the basis for the recommendation to consider modification of the influenza B virus composition of influenza virus vaccine to be produced for use in the United States during the 1966-1967 influenza season.

II. Investigations in volunteers of general phenomenon of immunity to influenza.

For more than thirty years it has been known that in naturally acquired and experimentally induced influenza,disease occurs in persons with moderate or high serum levels of neutralizing antibody directed against the infecting influenza virus while others with low titers of such antibody may escape infection. In a series of experiments in volunteers these circumstances were reproduced: subjects with substantial titers of antibody to 3 different influenza strains, including that used for live virus challenge were among those who became ill after challenge and those with low titers of only homotypic antibody escaped illness. A thesis to explain these findings and to relate them to the general problem of immunity in influenza and to the specific problem of continual change in antigenic composition of influenza viruses was proposed in a detailed report which was published.

III. Studies on influenza in immunologic virgin populations.

An epidemic of type A2 influenza was studied in three isolated Pacific atoll populations, in which almost no one had pre-epidemic neutralizing antibody to any of seven strains of type A and B influenza virus. The epidemic was carried to the atolls by ship, and involved nearly 100 percent of the populations at risk, with a high morbidity and a mortality in the most severely affected atoll of nearly 7 percent.

Following the epidemic, the highest neutralizing antibody titers occurred to the contemporary A2 strain, A2/Taiwan/1/64, with successively lower titers to earlier A2 strains (A2/Japan/170/62 and A2/Japan/305/57). Twenty-three percent of the tested individuals also showed rises to representative strains of heterologous A or A2 influenza viruses, mostly at low titers. The results which provide additional data on the dynamics of continual change in the antigenic composition of influenza viruses were described and interpreted in detail in a published report.

IV. Studies on viruses recovered from and capable of producing in man illnesses indistinguishable clinically from influenza.

In 1948, Pellissier and Lumaret (Ann. Inst. Past., 74: 507, 1948) isolated a virus in guinea pigs from a patient in Ubangi with African epidemic icterus. Later, Pellissier (Allgemein Path. u. Bakt., 16:483, 1953) recovered strains of the same virus from patients suffering from an anicteric, grippal or influenza-like illness. The recovered viruses when put into suckling mice induced signs and pathologic changes markedly similar to those induced in this animal by murine hepatoencephalitis virus. Studies were carried out to collect information to differentiate serologically epidemic icterus virus from myxoviruses. Antigens were prepared with 5 different virus strains (hepatoencephalitis strains MHV-11, -12, -13, -14 and epidemic icterus virus) from the brains or livers of infected suckling gnotobiotic mice. Antisera were prepared against 3 of the strains (MHV-11, MHV-12 and epidemic icterus virus) in adult gnotobiotic mice. The results of complement fixation tests with these reagents showed that antiserum prepared against each of the 2 hepatoencephalitis strains reacted with all of the hepatoencephalitis antigens; the epidemic icterus antiserum reacted with epidemic icterus, MHV-12,-13, and -14 antigens, but not with the MHV-11 antigen; the epidemic icterus antigen reacted with epidemic icterus and MHV-12 antisera, but not with the MHV-11 antiserum. The antigenic cross reactions obtained in these tests indicated that the African virus is a new, but distinct, member of the hepatoencephalitis virus family. The findings further suggest the possibility that infection with epidemic icterus virus or a closely related virus might account for the frequent presence of hepatoencephalitis virus antibody in human sera.

With the identification of epidemic icterus virus as a member of the hepatoencephalitis group, another family of viruses is added to the list of viruses capable of producing in man an illness which clinically might be mistaken for influenza.

V. Application of the Technicon Auto-Analyzer to influenza virus CCA measurement.

The automatic continuous flow technique for separation of agglutinated from non-agglutinated red cells developed by McNeil for blood typing was modified and applied to the automation of measurements of the hemagglutinin content of influenza virus vaccines and to the virus suspensions from which the vaccines are prepared. The description of the development phases of the modification of the instrument and of the application of the modified instrument to the measurement of myxovirus hemagglutinin were published. The described instrument and its application has now been adopted for use to measure the hemagglutinin content of myxovirus preparation in laboratories in this and other countries.

VI. Studies concerned with improvement of assay procedures to measure potency of influenza virus vaccines.

Inactivated influenza virus vaccines conventionally are assayed for potency in antigen extinction tests employing a measurement of neutralizing antibodies in the sera of vaccinated mice. However, it is now established largely from the work of Fazekas and others as well as from our work in volunteers, that antibody circulating in the vascular system is not directly involved in protection against clinical influenza. Influenza virus selectively adsorbs to the susceptible mucosal surface without entering the blood. This was shown clearly in studies performed in volunteers. Nevertheless, there is still very little information on the mechanism by which antibody reaches the respiratory surfaces. This means that the basic mechanism of immunity in influenza is not understood and further, this means that a determination of the serum antibody level of a vaccinated mouse might not be the most desirable measurement in assays to determine the potency of influenza virus vaccines. Rather, the most meaningful measurement might be the determination of the ability of mice vaccinated in antigen extinction tests to resist direct intranasal virus challenge. Accordingly, experiments are now underway to determine in vaccinated mice the degree of resistance to direct challenge and to relate these findings to results obtained in mice vaccinated by the conventional assay procedure and to observations in volunteers selected to participate in influenza virus vaccine assay tests.

VII. Characterization of viruses encountered in test animals and cell cultures employed in assays of potency of respirovirus vaccines.

As reported in the Annual Report 1964, a syncytium-producing virus was recovered from the visceral tissues of a rabbit. During the current year studies concerned with determination of the physical and biologic properties of the virus were completed, the findings were published and the virus distributed to interested investigators.

VIII. Viruses resistant to the action of chemical and physical agents. Scrapie virus which first attracted wide interest when it was found to be a contaminant in a lot of louping-ill vaccine is a virus of unusual resistance to the action of chemical and physical agents. Results of work carried out in the period 1959-1964 show clearly that this virus after its experimental introduction into mice becomes an agent of contagious infection for this animal. These findings raise the important question as to whether or not this virus is pathogenic for man. This question is of significance in vaccine production and testing since it is known that scrapie virus is present in the tissues of scrapie infected sheep and these animals might serve as a source of cell culture reagents for use in vaccine production and testing. The findings in mice were described and interpreted in a published report.

Proposed Course of the Project: Change in the antigenic composition of influenza viruses constitutes a problem of first importance in maintenance of adequate control of influenza by vaccination. This requires continuation of the established world-wide surveillance of influenza virus to detect soon after their appearance new influenza virus variants.

Nearly 1000 people who constitute the population of several atolls in the Caroline Island group escaped infection during the 1964 epidemic of influenza virus type A2. Subsequently, these people were shown by serologic tests to be dangerously susceptible to both type A and B influenza and therefore offered a unique opportunity to evaluate in a population of all ages the antibody response to influenza virus vaccines made from several different strains of virus. This study together with the determination of the persistence of antibody in the populations affected by the 1964 outbreak 18 months after the epidemic is now underway.

Laboratory differentiation of influenza and influenza-like-illnesses is an essential continuing study. Continuation of this study will make it possible to accumulate information that will be of assistance in eliminating or, at least, in reducing in importance a complicating factor in interpretation of influenza virus vaccine protection data and of other epidemiologic information related to influenza in man.

The definitive characterization of transmissible agents encountered in the tissues of animals used in the production or testing of vaccines will continue to be of assistance in overcoming manufacturing problems and in defining variables which are inherent in vaccine potency assay systems.

Honors and Awards: See next page.

Publications: See next page.

Honors and Awards:

Invited lecturer

- Scrapie seminar, U.S. Department of Agriculture, Hyattsville, Maryland, January, 1965.

- Workshop on slow, latent and temperate virus infections, National Institute of Neurological Diseases and Blindness, Bethesda, Maryland,January, 1965.

- Primary hepatoma: Ramifications on the etiology of atypical growth. National Advisory Cancer Council, Bethesda, Maryland, May 1965.

- Symposium on immunology and immuno-prophylaxis, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, December 1965.

Elected to membership

- Commission on Influenza, Armed Forces Epidemiological Board.

- Intercommission Committee on Standardization of Adenovirus Vaccines, Armed Forces Epidemiological Board.

- Temporary Committee for Formulation of Influenza Virus Vaccine for Use in Armed Forces in Influenza Virus Season, 1966-1967, Commission on Influenza, Armed Forces Epidemiological Board.

Program advisor and consultant

- Slow, latent, and temperate virus infection, National Institute of Neurological Diseases and Blindness, NIH.

Publications:

Morris, J. A., Jenkins, J. C., Horswood, R. L.: An automatic method for titration of influenza hemagglutinins. Annals New York Acad. Sci., 130: 801-808, 1965.

Morris, J. A., Saglam, M. and Bozeman, F. M. :Recovery of a new syncytium virus from a cottontail rabbit. J. Inf. Dis. 115:495-499, 1965.

Morris, J. A.: Viral etiology of hepatitis. In Primary Hepatoma. Ed. W.J. Burdette, Univ. of Utah Press, Salt Lake City, 1965.

Morris, J. A., Gajdusek, D. C. and Gibbs, C. J.: Spread of scrapie from inoculated to uninoculated mice. Proc. Soc. Exp. Biol. and Med., 120: 108-110, 1965.

Morris, J. A.: Early development in monkeys of cutaneous resistance to re-infection with Rickettsia tsutsugamushi. Proc. Soc. Exp. Biol. and Med., 119:736-738, 1965.

Morris, J. A.,: Mouse hepatitis (murine hepatoencephalitis) in Textbook of Diseases of Animals. Ed. R. J. Flynn. (In press).

Gibbs, C. J., Gajdusek, D. C., Morris, J. A. :Viral characteristics of scrapie agent in mice. Proc. of Workshop on Slow, Latent and Temperate Virus Infections, NINDB, Nat. Inst. Hlth. (In press).

Gibbs, C. J., Gajdusek, D. C., Morris, J. A. :Further observations in mice following inoculation of scrapie goat and sheep brain material. Proceedings of Scrapie Seminar, U.S. Dept. of Agriculture, U.S. Government Printing Office. (In press).

Knight, V., Kasel, J. A., Alford, R. H., Loda, F., Morris, J. A. :New research on influenza: Studies with normal volunteers. Annals of Int. Med. 62:1307-1325, 1965.

Morris, J. A., Kasel, J. A., Saglam, M., Knight, V. and Loda, F. A.:Immunity to influenza as related to antibody levels. New Eng. J. Med. 274:527-535, 1966.

Brown, P., Gajdusek, D. C. and Morris, J. A. :Epidemic A2 influenza in isolated Pacific Island populations without pre-epidemic antibody to influenza virus types A and B, and the discovery of other still unexposed populations. J. Epid. 83:176-188, 1966.

Serial No. 98
1.Virology & Rickettsiology
2. Respiratory Virus
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies concerned with adenoviruses

Previous Serial Number: None

Principal Investigator: J. A. Morris, Ph.D.

Other Investigators:
Marilyn Bozeman, M.S. (WRAIR)
Paul Brown, M.D.(NINDB)
Edward Buescher, M.D. (WRAIR)
Joseph Casey, M.D. (NIAID)
Bernice Eddy, Ph.D.
Jose David-Ferreira, M.D., Ph.D.(Portugal)
Carleton Gajdusek, M.D. (NINDB)
George Jackson, M.D. (Univ. Chicago)
Gordon Meiklejohn, M.D.(Univ. of Colorado)
Joseph Metzger, M.D. (Japan)
John Parker, Ph.D. (Micro. Asso. Inc.)
Robert Peckinpaugh, M.D. (NAMRU-4)

Cooperating Units:
Walter Reed Army Medical Center
University of California Medical Center
Laboratory of Infectious Diseases, NIAID
Gulbenkian Foundation (Portugal)
Laboratory of Child Growth and Development, NINDB
University of Chicago
University of Colorado
406th Medical General Laboratory (Japan)
Microbiological Associates, Inc.
NAMRU-4

Man Years:
Total: 1.3
Professional: 0.3
Other: 1.0

Project Description:

Objectives: The objective of this project is the accumulation of information which will be of help in re-establishing adenovirus vaccines to the armamentarium of respirovirus vaccines for use in man, and, in addition, will be of assistance in understanding the biology of adenovirus infection.

Investigation of biologic behavior of vaccine strains of adenoviruses. Two lots of adenovirus type 3 which were employed as seed stocks in the manufacture of adenovirus vaccines were demonstrated to have incorporated SV40 genetic material into the adenovirus capsid. Both lots were propagated in their early passage history in rhesus monkey kidney cell cultures known to be contaminated with SV40. Further passages were carried out in African green monkey kidney cell cultures in the presence of SV40 antiserum. Following this procedure, 2 separate passage lines were free of detectable infectious SV40; both lots induced in hamsters tumors that contained complement fixing antigens identical to those found in SV40 tumors. These tumors, in turn, elicited serum antibodies that fixed complement with SV40 tumor and T antigen. In addition, both lots induced formation in human embryo cells SV40 antigen which was detected by immunoflorescent techniques. The induction of fluorescent stainable SV40 T antigen in cell cultures was prevented by treatment of the virus with adenovirus type 3 antiserum, but not by treatment with SV40 neutralizing antiserum. Thus, the SV40 genetic material was presumably enclosed within adenovirus capsids. A full interpretative report of these findings is in press.

Proposed Course of the Project. In the course of the work concerned with the biologic behavior of adenovirus vaccines it was found that a small number of people possess in their prevaccination sera SV40 neutralizing antibody. These persons provided the opportunity to carry out work that is now underway to obtain information to determine whether or not the serum level of SV40 neutralizing antibody increased following vaccination with vaccines that contained adenovirus strains known to be "hybridized" with SV40 genetic material. Further, sera obtained from the same individuals will be examined for presence of antibodies directed against adenovirus T and tumor antigens and against the "small virus-like particle" antigen. Small virus-like particles are known to contaminate the vaccines employed in the study.

Honors and Awards: None

Publications:

Morris, J. A., Casey, M. J., Eddy, B. E., Lane, W. T., Huebner, R. J.: Occurrence of SV40 neoplastic and antigenic information in vaccine strains of adenovirus type 3, 1966. Proc. Soc. Exp. Biol. & Med. (In press).

Serial No. 62
1. Virology & Rickettsiology
2. Basic Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Investigations concerned with human hepatitis

Previous Serial Number: Same

Principal Investigator: Joseph P. O'Malley, M.D.

Other Investigators: Ruth Kirschstein, M.D.
Zell A. McGee, M.D.

Cooperating Units: Laboratory of Pathology, DBS
Walter Reed Army Institute of Research

Man Years
Total: 2.6
Professional: 1.0
Other: 1.6

Project Description:

Objectives: The purpose of this project is (1) to develop suitable laboratory methods for cultivating and handling one or more of the etiologic agents of human hepatitis, and (2) to evaluate the importance to man of certain agents presumed to cause human hepatitis.

Methods Employed: The tissue culture methods of a virus research laboratory are employed in this work. In studies on the A-1 agent, particular use is made of a plaque counting technique with chick embryo monolayers serving as the host system for isolation attempts and neutralization tests.

Major Findings: The study to determine the susceptibility of rhesus, cynomolgus, vervet and patas monkeys to infection with the A-1 agent has been completed. No signs of overt clinical disease developed in any of the animals tested. Moreover, serial liver biopsies taken over a 6-month period failed to reveal the development of any specific histopathological lesions. These animals do not appear to be susceptible hosts for the A-1 agent.

Over 2000 serum specimens from 700 persons with liver diseases other than viral hepatitis have been tested for A-1 plaque-reducing antibody. Less than 5% of this group of patients showed the presence of any A-1 antibody. Most of the sera containing A-1 antibody were borderline positives.

Observations have been made recently which indicate that studies with the A-1 agent may take a new direction. However, until these observations are investigated further and developed in more detail, a proper evaluation cannot be made. These developments should be available for enumeration and discussion in the next report.

Significance to Bio-Medical Research and the Program of DBS: The threat of epidemic hepatitis that constantly jeopardizes the health of both civilian and military populations, coupled with the hazard of contamination of whole blood and blood derivatives with serum hepatitis virus, makes the control of these diseases one of the major unresolved problems of contemporary virology.

Proposed Course of the Project: In the coming year additional emphasis will be placed on developing new approaches to the problem of isolating microbiologic agents from known and suspect icterogenic materials and specimens from persons with clinical hepatitis. The recent developments relating to the A-1 agent will be evaluated and interpreted as soon as possible. The subsequent course of the project will hinge on the resolution of these findings.

Honors and Awards: None.

Publications: None.

Serial No. 59
1. Virology & Rickettsiology
2. Basic Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Antigenic studies of epidemic typhus rickettsiae

Previous Serial Number: Same + 58

Principal Investigator: Lewellys F. Barker, M.D.

Other Investigators: J. K. Patt, M.S.
A. E. Palmer, D.V.M.
R. L. Kirschstein, M.D.

Cooperating Units: Laboratory of Pathology, DBS

Man Years:
Total: 2.5
Professional: 1.0
Other: 1.5

Project Description:

Objectives: The objective is to investigate the antigenicity and the immunogenicity of three fractions of epidemic typhus rickettsiae and to attempt to localize the protective antigens for further study. The fractions are the soluble antigen, the cell wall and the intracellular protoplasm.

Methods Employed: (A) Rickettsiae are purified from infected yolk sacs by differential centrifugation, treatment with celite, albumin and trypsin and sucrose gradient centrifugation. Disruption of the organisms is done with a Mickle apparatus. The cell walls are then separated from intact rickettsiae by sucrose gradient centrifugation. After purification, the cell walls and intact rickettsiae are examined with the electron microscope. Soluble antigen is obtained by ether extraction of infected yolk sac suspensions. Purification of rickettsiae from heavily infected tissue cultures is accomplished by a low and a high speed centrifugation followed by a sucrose gradient centrifugation.

(B) Rhesus and vervet monkeys were inoculated by the subcutaneous and intravenous routes with varying doses of Breinl and Strain E rickettsiae. Temperatures were recorded daily and the animals were observed for signs of illness. Hematology, serology and isolation studies were performed using routine methods.

Major Findings: (A) A summary of the results of repeated determinations on a number of preparations is presented in Table 1. The fractions studied showed little difference in their ability to fix complement. In the mouse protection test, the intact rickettsiae and soluble antigen were more potent than the cell walls or protoplasm. Soluble antigen was the most effective material in assays of guinea pig protection against fever following a massive challenge. Both cell walls and intact rickettsiae showed threefold greater protection on a weight basis than the protoplasm.

Tests of specificity with murine and epidemic typhus antisera revealed greater specificity in the protoplasm than in any other fraction. Hyperimmunization of rabbits, Ouchterlony and CF tests all revealed that none of the fractions was entirely free of cross reactions with egg material. The soluble antigen, which is made by ether extraction of unpurified rickettsiae, contains the largest amount of egg material, as is to be expected from the method of preparation.

(B) In an effort to find an alternative to the febrile reaction in guinea pigs infected with massive doses of epidemic typhus rickettsiae for studying protective immunity, the susceptibility of rhesus and African green monkeys to epidemic typhus was evaluated. Both species were readily infected by subcutaneous or intravenous inoculation, but a severe illness occurred only after inoculation of massive numbers of rickettsiae intravenously. Persistence of rickettsiae in the animals' tissues up to 5 weeks after inoculation was noted. Also, typical typhus nodules in the central nervous system were found in many of the animals when they were sacrificed.

Significance to Bio-Medical Research and the Program of the Division. Commercial epidemic typhus vaccine is a crude product containing a large quantity of egg material as well as intact and partially degraded rickettsiae. As a vaccine ideally should contain only the antigen(s) needed to evoke protective immunity, it is important to look for these antigens in complex microorganisms such as rickettsiae.

Proposed Course of Project. This project will continue to focus on the three major fractions mentioned under Objectives. Because of the difficulty in purifying rickettsiae from infected yolk sacs, emphasis will be placed on obtaining rickettsial antigens from tissue culture grown suspensions. Several pilot lots of partially purified epidemic typhus vaccine will be prepared from infected yolk sacs and evaluated with regard to potency and stability.

Honors and Awards: None

Publications: None

Serial No. 59

Table 1. Antigenicity and Immunogenicity of Fractions of Epidemic Typhus Rickettsiae

Antigen	Mean complement fixing antigen unit (ug per 0.25 ml)	Mean mouse protection unit (ug per PD_{50})	Mean guinea pig protection unit (ug per PD_{50})	Protein content (folin)
Intact rickettsiae	2.1	0.052	10	50%
Cell walls	4.0	0.161	10	20 - 40%
Protoplasm	2.0	0.653	31	30%
Soluble antigen	1.7	0.025	3.1	60%

Serial No. 63
1. Virology & Rickettsiology
2. Basic Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1,1965 through June 30, 1966

Project Title: Development of continuous tissue culture lines for use in testing of vaccines

Previous Serial Number: Same

Principal Investigator: Hope E. Hopps, M.S.

Other Investigators: Barbara C. Bernheim, M.A.

Cooperating Units: None

Man Years:
Total: 1.2
Professional: .6
Other: .6

Project Description:

Objectives: To establish cell lines or strains suitable for laboratory testing of vaccines.

Methods Employed: Modern tissue culture techniques of cultivation and maintenance were employed.

Major Findings: (1) Cercopithecus kidney: In an effort to establish a "back-up" cell line for BS-C-1, particularly for use in measles vaccine testing, a second line of Cercopithecus kidney is under study. During the past year 61 parallel titrations of a standard pool of measles virus have been carried out in BS-C-1 and BS-C-2 cells. In the BS-C-1 line, the titers ranged from $10^{-1.81}$ to $10^{-4.1}$ $TCID_{50}$/ml with an average of $10^{-2.21}$ and in the BS-C-2 line, from $10^{-2.3}$ to $10^{-4.3}$ with an average value of $10^{-2.47}$. Thus, it appears that a suitable cell line is available for substitution when necessary. During the past year, the BS-C-1 line has been sent to 23 laboratories in the U.S. and abroad.

(2) Mouse embryo, guinea pig kidney, patas kidney: Each of these strains, developed in this laboratory, is to be examined periodically for susceptibility to such agents as SV40, measles, polio and rubella viruses.

Significance to Bio-Medical Research and the Program of DBS: The development and testing of cell lines free of adventitious agents is important

to virus vaccine testing programs.

Proposed Course of Project: This is a continuing project in which lines important to the laboratory will be maintained; as other lines are developed they will be examined for their susceptibility to such agents as SV40, measles and rubella as well as for their usefulness in studies with Mycoplasma and rickettsiae.

Honors and Awards: None

Publications: None

Serial No. 65
1. Virology & Rickettsiology
2. Basic Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Mechanisms of intracellular infection

Previous Serial Number: Same + 64

Principal Investigator: Hope E. Hopps, M.S.

Other Investigators: Lewellys F. Barker, M.D.
Barbara C. Bernheim, M.A.

Cooperating Units: Laboratory of Viral Immunology, DBS
Laboratory of Bacterial Products, DBS
Laboratory of Viral Oncology, NCI
Arthritis and Rheumatism Branch, NIAMD

Man Years:
Total: 2.3
Professional: 1.3
Other: 1.0

Project Description:

Objectives: The main objective of this continuing project is the accumulation of information concerning the mammalian host cell and its response to infection with rickettsiae, viruses and PPLO. The present studies under this project are concerned with:

(1) Production of rickettsial interferon in vivo.

(2) A comparison by electron microscopic observation of intracellular mycoplasma, larger viruses and rickettsiae.

(3) PPLO-host cell interaction.

(4) Rubella virus - host cell interaction

(5) Attempted isolation of psittacosis group agents from specimens, (joint fluids, etc.) obtained from clinically diagnosed arthritis cases.

(6) Isolation of a possible psittacosis agent from Cercopithecus aethiops.

Methods Employed: Modern tissue culture techniques and the standard procedures used in a research laboratory engaged in studies on viral and rickettsial agents were employed.

Major Findings: (1) Rickettsial interferon. Certain rickettsiae do induce interferon formation in tissue culture systems; however, nothing is known concerning interferon production in vivo. During the past year studies have been carried out with the avirulent E and virulent Breinl strains of epidemic typhus (Rickettsia prowazeki). Guinea pigs and mice were injected intravenously with high concentrations of rickettsiae and serum samples were taken at 0,1,2 and 3 hrs after injection, a time at which in vivo viral-induced interferon has been detected. However, rickettsial interferon was not observed with either mouse or guinea pig sera. In addition, serum specimens from rhesus monkeys infected with typhus organisms yielded negative results. From the present data, at least, it does not appear that interferon production is a prominent feature of rickettsial proliferation in vivo.

(2) Comparison of intracellular Mycoplasma, larger viruses and rickettsiae. Since it has been suggested that certain structural similarities exist between Mycoplasma, larger viruses and rickettsiae, this now-completed project was undertaken to study these 3 groups of organisms under rigidly comparable biological and biophysical conditions. In these studies each agent employed was propagated in BS-C-1 cells under identical conditions and such cells were then subjected to standardized fixation and embedding procedures prior to examination by elution microscopy. The organisms studied were R. rickettsii (Rocky Mt. Spotted fever), R. prowazeki (obtained from Japan), the P-4 strain of ornithosis and Mycoplasma hominis, type 1. Each of the rickettsial species examined had a cell wall and a plasma membrane, and contained ribosomes and deoxyribonucleic acid (DNA) in a ground substance. However, certain differences were noted. Both strains of R. prowazeki contained numerous intracytoplasmic electron-lucent spherical structures (4 to 10 mu), not previously described. R. sennetsu, unlike the other rickettsiae, was not free in the host cytoplasm but was always enclosed in a vacuole. R. rickettsii was observed intranuclearly and in digestive organelles of the host cell as well as in the cytoplasm. Cells infected with ornithosis virus contained several forms representing the stages in its life cycle. The "initial bodies", made up of ribosomes and DNA strands, were morphologically similar to the rickettsiae. In cultures infected with M. hominis, most of the cells became large and multinucleate. Although the Mycoplasma organisms were readily cultivated from these cultures, only a few could be found in the electron microscope preparations. These organisms were extracellular and lacked a cell wall, being bound only by a unit membrane. Again, the internal components were ribosomes and DNA strands. Under the uniform preparative conditions employed here, the three groups of organisms were morphologically distinguishable from one another.

(3) PPLO-host cell interaction. When stationary cultures of Mycoplasma-infected RK13 cells were treated with kanamycin, the infection persisted despite prolonged treatment. However, in RK13 cultures growing in spinner flasks it was possible to eliminate the Mycoplasma permanently. Similar results were obtained when BS-C-1 cultures infected with Mycoplasma hominis, type 1 were so treated. Since this reliable "curing" method was developed, collaborative studies were recently initiated to determine any changes in the

"cured" cells. Using homology techniques, the DNA from "cured" BS-C-1 cells will be examined for its relatedness to M. hominis DNA.

(4) Rubella virus-host cell interaction. In the search for laboratory markers which could be used in determining rubella virus attenuation, attention was directed to the interferon-inducing capacities of 2 types of rubella virus, one which had been subcultured 4 times in BS-C-1 cells and one which had undergone 76 consecutive passages in primary African green monkey kidney cells (GMK). Fluids harvested from GMK cultures infected with low (4th) and high (76th) passage virus inhibited vesicular stomatitis virus (VSV) plaque formation. Quantitative time course studies of virus multiplication and interferon production revealed that although the virus titers in each system were essentially identical, the titer of interferon induced by high passage virus was 2-4 fold greater than that obtained with low passage virus. The consistent quantitative difference was observed in 6 separate experiments. A gradual shift to higher interferon titers was observed in cultures infected with virus stocks harvested at passage levels 2, 10, 20, 37, 52 and 66. The data indicate that increased interferon production can be reliably used as one marker for rubella virus attenuation.

(5) Attempted isolation of psittacosis-group agents from joint fluids obtained from arthritis cases. It has been suggested that an infectious agent, possibly a member of the psittacosis group, may be involved in Reiter's syndrome. In collaboration with Dr. John Decker, NIAMD, isolation attempts are being made in available clinical material. This project was recently initiated and no results are known at present.

(6) Isolation of a psittacosis-like agent from Cercopithecus aethiops tissue culture material. This agent, which grows intracellularly in tissue cultures producing marked CPE was identified as a Mycoplasma species; no further studies with this agent are anticipated.

Significance to Bio-Medical Research and the Program of DBS: Basic studies of host-virus interaction are a necessary parallel to the development of systems useful for vaccine production and related testing procedures.

Proposed Course of Project: Sub-projects 2-5 will be continued during the coming year.

Honors and Awards: None

Publications:

Anderson, D. R., Hopps, H. E., Barile, M. F. and Bernheim, B. C.: Comparison of the ultrastructure of several rickettsiae, ornithosis virus and Mycoplasma in tissue culture. J. Bacteriol., 90: 1387-1404, 1965.

Serial No. 66
1. Virology & Rickettsiology
2. Basic Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on virulence and avirulence in rickettsiae

Previous Serial Number: Same

Principal Investigators: Hope E. Hopps, M.S.
Lewellys F. Barker, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.0
Professional: .6
Other: .4

Project Description:

Objectives: The overall objective of this project is to study the characteristics of virulent and avirulent rickettsiae from the typhus, spotted fever and scrub typhus groups.

Methods Employed: Modern tissue culture and embryonated egg techniques and the standard serologic procedures used in studies on viral and rickettsial agents were employed.

Major Findings: Comparison of strain E and Breinl in Tissue Culture. Strain E growth is slower than Breinl growth at 39° as well as at 35°. Both strains multiply at 39°, however, and growth of both strains at 39° is slower than at 35°. No difference in rate of initial infection, or penetration of cells was noted between the two strains. The progress of infection by both strains is showed by the presence of immune serum in the maintenance medium. No difference in susceptibility to chloramphenicol, 1 ug/ml, was noted between the two strains. Both strains multiplied with as little as 2% calf serum in the maintenance medium, but multiplication of each strain occurred more rapidly with 20% calf serum in the maintenance medium.

Titration and neutralization of scrub typhus in tissue culture. Titration of the Karp, Gilliam and Kato strains in BS-C-1 cells yield endpoints comparable to those obtained in mice. Each strain produces a distinctive CPE in tissue culture. Neutralization of each strain can be demonstrated by incubation with homologous hyperimmune serum prior to inoculation of cell

cultures.

Significance to Bio-Medical Research and the Program of DBS: Increased interest in the use of attenuated vaccines makes necessary a better understanding of those factors which influence virulence and avirulence.

Proposed Course of Project: Further efforts to distinguish between strain E and Breinl growth in tissue culture will be made in the presence of agents which will either enhance or inhibit growth.

Studies are planned to elucidate the mechanism of neutralization of rickettsiae in tissue culture by antiserum. A search for similar interaction between antiserum and microorganisms is planned with the psittacosis LGV - trachoma group.

Honors and Awards: None

Publications: None

Serial No. 82
1. Virology & Rickettsiology
2. Basic Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies of the nucleic acids and genetic relatedness of rickettsiae

Previous Serial Number: None

Principal Investigator: Richard A. Mason, D.V.M., Ph.D.

Other Investigators: Emilio Weiss, Ph.D.

Cooperating Units: Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.

Man Years
Total: 1.1
Professional: 0.8
Other: 0.3

Project Description:

Objectives: The purpose of this project is to compare the genetic relatedness of various rickettsiae and other selected intracellular parasites and to characterize rickettsiae with respect to the important criterion of DNA base ratios.

Methods Employed: Initial studies were done with Rickettsiae quintana because this parasite can be propagated on agar. In preparation for genetic studies by means of the DNA homology technique, isotopically labeled nucleic acid precursors were investigated for their ability to be incorporated into DNA. DNA base ratios were studied by standard methods of thin-layer chromatography and by the simpler melting curve procedure.

Major Findings: DNA was obtained from R. quintana in good yield by means of a simple procedure. Such DNA had a molecular weight suitable for homology studies and contained little protein as indicated by U-V absorption and chemical procedures. The content of DNA in R. quintana was consistent with DNA values given in the rather meager number of reports concerning rickettsial nucleic acids.

Several compounds were tested for incorporation into DNA, including cytidine, deoxycytidine, cytosine, uracil, uridine, adenine, thymine, thymidine and ribose. Under the experimental conditions employed, none was used in DNA synthesis. However, it was possible to label rickettsial DNA with glycine C-14 thus providing the means for carrying out the planned genetic studies.

DNA from R. quintana contained only the four standard bases and in this respect appeared qualitatively similar to DNA from other rickettsiae. More importantly, the base ratios were similar to DNA values determined for a limited number of rickettsiae some years ago by more laborious techniques.

Significance to Bio-Medical Research and the Program of DBS: Studies of rickettsial genetics and DNA are of importance as an additional means of understanding relationships among rickettsiae and in discovering features-in-common between these microorganisms and other intracellular parasites. Studies of DNA precursors are of significance to rickettsial physiology.

Proposed Course of Project: This is a continuing project. Several rickettsiae and other microorganisms will be studied during the next year.

Honors and Awards: None

Publications: None

Serial No. 67
1. Virology & Rickettsiology
2. Tissue Culture
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on malignant cell transformation in vitro

Previous Serial Number: Same

Principal Investigator: Hector Montes de Oca, M.D.

Other Investigators: None

Cooperating Units: None

Man Years
Total: 0.6
Professional: 0.2
Other: 0.4

Project Description:

Objectives: To study patterns of malignant transformation in cultures of various animal cells, especially those of potential importance in vaccine production.

Methods Employed: Animals, pre-conditioned by immuno-suppressive treatment, are inoculated by different routes with various generation levels of newly established serially propagated cell cultures. The inoculated animals are then observed for tumor development. Cells not known to transform spontaneously (e.g., WI-38) were treated with successive doses of X-rays or dimethylbenzanthracene to obtain transformation.

Major Findings: To study patterns of spontaneous transformation, a cell line of subcutaneous rat tissue origin was initiated and cells were reinoculated into the donor animal to determine tumorigenic potential. No tumors were obtained from cells at passage levels 1 through 15, at which time the cell cultures were frozen for storage in the cell bank. The tumorigenicity of the WI-38 cell strain at 25th passage level was determined by inoculating these cells both into the cheek pouch of hamsters pre-conditioned with high doses of X-ray and into suckling hamsters by the intracerebral route. No tumors were obtained by either method, thus confirming similar reports of other investigators. Chromosome counts are being performed on X-irradiated WI-38 cells. Preliminary observations indicate an increase in the frequency of heteroploid cells.

Significance to Bio-Medical Research and the Program of the Division: Interest in the potential usefulness of diploid cells in vaccine production makes necessary a study of their biologic properties, particularly with regard to malignant transformation.

Proposed Course of Project: This is a continuing project. The studies of spontaneous transformation patterns and of WI-38 tumorigenic capacity will be pursued as available assistance allows; transformation experiments with WI-38 cells with x-rays and chemical carcinogens will be actively pursued.

Honors and Awards: None

Publications: None

Serial No. 79
1. Virology & Rickettsiology
2. Tissue Culture
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Development of new cell culture systems and techniques

Previous Serial Number: Same

Principal Investigator: Hector Montes de Oca, M.D.

Other Investigators: Robert E. Grubbs

Cooperating Units: TC Section, Laboratory of Biology, NCI

Man Years
Total: 0.7
Professional: 0.2
Other: 0.5

Project Description:

Objectives: To develop new cell culture systems and to modify or develop new cell culture techniques as needed by this Section in order to cope with the new developments relevant to the mission of the Division.

Methods Employed: Standard and experimental procedures for the cultivation of primary animal cells and heteroploid and diploid cell lines.

Major Findings: (1) Preparation of primary cultures. Systematic studies of the trypsinization procedure were performed leading to a modification (a combination of 0.02% versene and 0.25% trypsin) that resulted in the yield of cells per gram of tissue from 70×10^6 in 1964, to 150×10^6 in 1965. (a summary of these results will be presented at the meeting of the Tissue Culture Association in June 1966); (2) Development of laboratory methods and techniques. Tissue culture media are now made from commercially compounded powdered materials, thus permitting preparation and standardization of large batches sufficient for six months' use. This procedure has resulted in an 87% decrease in cost per liter (from $5.00 to $0.65); (3) Development of new cell lines suitable for the purposes of DBS: a) a cell line of rhesus monkey kidney BSTC224 is being developed in the laboratory; the history and rate of growth permits forecast that it will be a permanent line; b) recent attempts to develop a RIF-free chick embryo cell line were not successful; however, a detailed study of the exact nutritional requirements of chick cells is planned; (4) Freezing of cell lines:573 ampules representing 31 different cell lines of interest to the DBS cell users were frozen in liquid nitrogen during 1965; (5) Hormone action in chemically defined medium: These studies were done in collaboration with the Tissue Culture Section,

Laboratory of Biology, National Cancer Institute. The action of triiodothyronine on NCTC 2071 cells in chemically defined medium (NCTC 135) is being studied. Preliminary experiments indicate an apparent growth promoting action of this hormone; (6) Suspension cultures. Attempts to grow suspension cultures of WI-38 cells were not successful so far (studies in collaboration with TC Section, LB, NCI).

Significance to Bio-Medical Research and the Program of DBS: The production and maintenance of cell cultures as well as the development of new tissue culture methods are of paramount importance to the control and research mission of the Division.

Proposed Course of Project: This project will be continued in its several aspects: (1) Emphasis will be placed on changes in the physical facilities of the laboratory in order to improve the sterility of working conditions. The effectiveness of Laminar Flow clean air bench units will be investigated; (2) Semi-automatic methods for preparing primary cultures will be tried; (3) Studies to develop a RIF-free chicken embryo cell strain will be pursued. Attempts will be made to establish other strains of potential interest; (4) The liquid nitrogen freezing facilities will be enlarged. Sufficient quantities of cell lines and strains that are in great demand will be frozen in large amounts; thus, if contamination occurs, it will be possible to resume production in less than one week; (5) The growth promoting action of different hormones will be studied; (6) The possibility of growing WI-38 cells in suspension will be pursued.

Honors and Awards: None

Publications: None

Serial No. 83
1. Virology & Rickettsiology
2. Tissue Culture
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Antigenic study of WI-38 cells

Previous Serial Number: None

Principal Investigator: H. Montes de Oca, M.D.

Other Investigator: Robert M. Pennington

Cooperating Units: Laboratory of Blood & Blood Products, DBS

Man Years
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: To determine the presence of blood group or tissue transplantation antigens in WI-38 cells.

Methods Employed: Antibody reagents are being prepared by intravenous inoculation of WI-38 cells into mice previously treated intraperitoneally with adjuvants. The ascitic fluid thus produced will be tested for the presence of human blood group antibodies.

Major Findings: Forty animals were repeatedly inoculated over a period of 8 months and 70 ml of ascitic fluid with high WI-38 cell agglutination titers were obtained. After absorption to remove species antibodies, preliminary tests show the presence of an antibody which remains to be identified.

Significance to Bio-Medical Research and Program of DBS: The possibility that WI-38 cells might be used for vaccine production required the determination of its antigenic capacity. The sensitization of the recipient of this vaccine with human antigens could possibly be a health hazard.

Proposed Course of Project: This is a continuing project. The necessary tests for characterizing the antigen will be performed. Coombs' mixed agglutination test will be performed with WI-38 cells using different blood group testing serums.

Honors and Awards: None

Publications: None

Serial No. 72
1. Virology & Rickettsiology
2. Basic Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Comparative studies on the newer detection and assay systems for the viruses of avian leukosis complex

Previous Serial Number: Same

Principal Investigator: Alexis Shelokov, M.D.

Other Investigators: Nicola M. Tauraso, M.D.
Calvin G. Aulisio, M.S.
William G. Jahnes

Cooperating Units: Virus Vaccine Testing Unit, Lederle Laboratories

Man Years:
Total: 2.8
Professional: 1.3
Other: 1.5

Project Description:

Objectives: To compare the relative effectiveness of the RIF, COFAL and fluorescent antibody (FAB) techniques for the detection and assay of several members of the avian leukosis complex of viruses.

Methods Employed: This study is a cooperative effort of three groups of investigators within LVR. The Avian Leukosis Unit performs the RIF tests according to the standard DBS procedure used in the testing of vaccines; the Immunoserology Unit performs the COFAL test (complement-fixation for avian leukosis); the FAB facility performs the fluorescent antibody test. Antibody and antigen reagents are prepared and standardized for use in these studies; other techniques are utilized as required, such as cell culture, animal and egg inoculation with viruses, and tumor transplantation.

Major Findings: Individual records of tests performed are summarized in Tables 1, 2, 3 and 4. The number of RIF tests performed is reported under a separate project title, "Avian Leukosis viruses in relation to human vaccines." (Report No. 73)

The overall results in the comparative studies indicate that (1) close correlation exists between the RIF, COFAL and FAB tests, (2) the FAB test is the most sensitive in detecting minute amounts of antigen, (3) the RIF test is the least sensitive and becomes positive after 2 or 3 additional passages

are made, (4) the COFAL test is often able to detect antigen 1 passage level after the FAB test and 1 passage level before the RIF test. Occasionally, both the COFAL and FAB tests are positive at the same passage level.

Rubin and Kottaridis have reported the use of passively conferred antibody present in egg yolk for neutralizing Rous sarcoma and other avian leukosis viruses. Preliminary data from tests performed in our laboratory indicate that this maternal antibody can be successfully employed in the indirect FAB technique. A total of 40 egg-laying hens of which 4 had serum antibodies against members of the avian leukosis viruses were selected for this study. The eggs from the 4 hens with serum antibodies were also positive for maternal antibodies when examined by the FAB test. The remaining 36 hens were negative for passively conferred antibody.

The laboratory has continued to maintain 4 different strains of Rous sarcomas by passage in hamsters for the production of reagents necessary for performing both the COFAL and FAB tests. In addition, cell cultures have been initiated from all 4 tumors and studies are currently being performed utilizing these cell cultures and the hamster-derived tumor antigen and antibody reagents.

Significance to Bio-Medical Research and the Program of the Division: The RIF test is required on live virus vaccines prepared from chick embryo. A combination of the tests employed providesa better means for checking for the presence of avian leukosis viruses.

Because RIF-free chick embryo fibroblasts are required in the safety testing of vaccines, a RIF-free chicken flock must be maintained at NIH. The monitoring of this flock can be rapidly and easily achieved by using techniques to test eggs for maternal antibody by the indirect FAB technique.

Proposed Course of Project: This cooperative study will be continued.

Honors and Awards: None

Publications: None

Report for Calendar Year 1965

Table I

Number of fluorescent antibody tests performed

Material tested and processed	No. tested	No. positive for virus and/or antibody
RIF-test coverslips	1784	459
Chicken serum	1044	48
Egg yolk	1036	78
Total	3,864	585

Table II

Number of COFAL tests performed

Material tested	No. tested	No. positive for virus and/or antibody
RIF-test cell cultures	665	373
Hamster sera	1017	60
Total	1682	433

Table III

Number of Rous sarcoma tumor transplants in animals

Rous-sarcoma tumor strain	No. of animals transplanted	No. of passages
Carr-Zilber	192	12
Prague	224	14
Bryan	288	18
Schmidt-Ruppin	240	15
Total	944	59

Table IV

Number of hamster-derived Rous Sarcomas in cell culture

Rous-sarcoma tumor strain	No. Passages
Schmidt-Ruppin (Flow)	5
Carr-Zilber (DBS)	6
Prague (DBS)	3
Schmidt-Ruppin (DBS)	2
Bryan (DBS)	3

Serial No. 73
1. Virology & Rickettsiology
2. Basic Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Avian leukosis viruses in relation to human vaccines

Previous Serial Number: Same

Principal Investigator: William G. Jahnes

Other Investigators: Alexis Shelokov, M.D.
Nicola M. Tauraso, M.D.
Calvin G. Aulisio, M.S.

Cooperating Units: Laboratory Aids Branch, DRS
Laboratory Infectious Diseases, NIAID

Man Years:
Total: 2.7
Professional: 1.2
Other: 1.5

Project Description:

Objectives: (1) To test live and inactivated viral vaccines prepared from chick embryo tissue or from chick embryo cells in tissue culture for the presence of Resistance-Inducing-Factor (RIF); (2) to develop new and more sensitive procedures for detecting the presence of viruses of the avian leukosis complex.

Methods Employed: Standard cell culture techniques and modifications, complement-fixation tests and fluorescent antibody tests.

Major Findings: During the 12 month reporting period a total of 147 RIF tests were performed; of these, 30 were tests on 23 live measles vaccines, which were found to be RIF-test negative. The remaining 117 were mostly RIF tests on eggs received from 5 different sources, including the "old" and "new" NIH flocks. Positive results were found in 18/72 completed tests. After the two chickens which were "intermittent virus shedders" were destroyed in the older NIH flock, no further positive egg tests or positive serological results were obtained on the hens and a new flock was established. This flock was found to be antibody-free after 3 consecutive tests, but tests on the eggs for virus showed at least one intermittent RIF virus shedder which was then isolated. Egg tests during the last few months have been consistently negative.

Concurrently with the RIF testing of all vaccines and egg pools, COFAL and FAB comparative tests were run on all passage levels. Results of the

comparative testing are included in another report. In the majority of cases, the three tests agreed as to whether RIF was present or absent. Dr. K. Cook , LID, NIAID, again collaborated in testing duplicate FAB slides to help confirm our test results. These tests showed that the FAB test is an excellent quick method for monitoring chicken flocks either for antibody or for virus from sera, or eggs, and is a valuable asset in screening vaccines for RIF.

Significance to Bio-Medical Research and to the Program of the Division: At present the RIF test is still the procedure of choice for detecting viruses of the avian leukosis complex. The development of more sensitive and more reliable procedures is of utmost importance to the Division's mission.

Proposed Course of Project: Work will continue to improve the present RIF test and to develop simpler and more rapid procedures for the detection of the avian leukosis complex viruses.

Honors and Awards: None

Publications: None

Serial No. 74
1. Virology & Rickettsiology
2. Virus Biology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on natural antiviral and antitumor agents (Paolins)

Previous Serial Number: Same

Principal Investigator: C. P. Li, M.D.

Other Investigators: B. Prescott, Ph.D., LBC, NIAID
B. E. Eddy, Ph.D., Section on Experimental Virology, DBS
C. W. Hiatt, Ph.D., LBB, DBS
E. C. Martino

Cooperating Units: Laboratory of Infectious Diseases, NIAID
Laboratory of Biophysics & Biochemistry, DBS

Man Years
Total: 4.5
Professional: 1.0
Other: 3.5

Project Description:

Objectives: To continue the study of the antitumor activity of clam extracts in two ways: (a) to confirm and extend our previous work on the antitumor activity of the clam extract in hamsters and other animals, and (b) to prepare an antitumor fraction (paolin III) in a reasonably purified form and in sufficient quantity for further experimentation and for possible clinical trials.

Methods Employed: Two kinds of water extracts, autoclaved (121°C., 30 minutes) and unautoclaved were used for the present study. The unautoclaved water extract has been partially purified and concentrated by various physico-chemical methods and the assay of these different products is not yet completed.

The antitumor activity was assayed in vitro and in vivo by 3 different techniques. A. In vitro cell culture test: The clam extract was added at the desired concentration to a freshly prepared suspension of testing cells such as HeLa cells (approximately 200,000 cells per ml). The cells were then distributed into roller tubes and incubated for 3 days. The degree and pattern of cell growth with or without CPE was observed. Similarly cultured cells, but not treated with clam extract, served as controls. B. In vitro L1210 leukemia cell test: A suspension of L1210 leukemia cells from the spleen of a mouse was incubated with the clam extract at the desired concentration for 1 hour at 37°C. The cells were then inoculated subcutaneously

into mice. Cells incubated merely with Hanks' solution served as control inoculum. The length of survival time of the mice indicated the degree of activity. C. In vivo L1210 leukemia cell test: A suspension of L1210 leukemia cells (not incubated) was inoculated subcutaneously into mice. Treatment of mice consisted of a series of 7 or 8 daily subcutaneous or intraperitoneal injections of the clam extract at various concentrations, starting 24 hours after inoculation. Control mice were not treated or treated only with saline. The length of survival time of the mice indicated the degree of activity.

Major Findings: (1) General prophylactic effect against tumor formation in hamsters induced by adenovirus 12. It was previously reported that when newborn hamsters were inoculated with adenovirus 12 and several days later were treated with a series of daily subcutaneous injections of the crude clam extract , the subsequent development of tumors in the treated hamsters was greatly inhibited as compared with the untreated control animals. This finding was confirmed with similar experiments in the present study. Using 22 litters, or 358 randomized hamsters in 4 separate experiments carried out at different times, the tumor rate in the treated hamsters was reduced to less than half of that in the untreated control animals during an observation period of 90 days. In one experiment, 18 out of 50 untreated hamsters showed tumors before the 46th day after virus inoculation while only 1 out of 24 treated animals showed a tumor on the 85th day. In another experiment the per cent of tumor rate for 24 untreated hamsters was 71 while that for 46 treated ones was 26.

The question is naturally raised whether this prophylactic effect on tumor formation is antiviral or antitumor in nature. In answering this question it must be mentioned that in the foregoing experiments both autoclaved and unautoclaved extracts were used for treatment and one was as effective as the other. In separate experiments it was found that the unautoclaved extract showed both antiviral activity in vitro and general or local therapeutic effect against transplanted tumors in hamsters or mice. The autoclaved extract on the other hand, showed no such antitumor effect in vivo but inhibited adenovirus 12 in human embryonic kidney cell culture. Therefore, the inhibition of tumor formation in hamsters by the autoclaved extract is probably due to some antiviral mechanism. The autoclaved extract might represent paolin II (antiviral) which was separated at a high temperature from the unautoclaved material, a mixture of both paolin II and paolin III (antitumor). However, we are aware of the possible alternative that the antiviral and antitumor activity is due to the same component and autoclaving merely reduced the activity quantitatively.

(2) Local therapeutic effect against solid subcutaneous tumors in hamsters and mice. When the unautoclaved clam extract is injected into and/or around primary or transplantable adenovirus 12 tumors or transplanted SV40 tumors, therapeutic effect was demonstrated. Usually 100 mg of the extract in a volume of 1 ml was given daily for 3 to 10 days for a tumor around 1.0 x 1.0 cm in size. In favorable cases, the tumor usually became swollen in 24-48 hours

after receiving the first injection of the clam extract. Later the overlying skin became moist. With continuing injections tumors shrank to a black eschar which eventually separated, leaving a clean, shallow ulcer. Complete recovery then occurred without signs of systemic toxicity within 5 weeks after first injection. In other cases, the tumors grew again after a brief period of retardation and the hosts eventually died.

Recurrence of one or more tumors either at the same site of the regressing tumor, or at some distant site, was sometimes observed. Some of these tumors were re-treated and among them about 50 percent responded and regressed while others grew and killed the host.

In a series of experiments 117 hamsters were used, 78 bearing primary or transplanted adenovirus 12 tumors and 39 transplanted SV40 tumors. Among the adenovirus 12 tumors, 50 were treated and 28 or 56% showed complete regression. (These included those hamsters showing recurrence of tumors but eventually recovered after further treatment) while all the 28 untreated tumors grew to large size, killing the host. Among the SV40 tumors 24 were treated, resulting in 66% complete regression. All the 15 untreated hamsters bearing SV40 tumors died. All the animals that recovered have been normal in appearance to date without further recurrence of tumors during the period varying from 2 to 5 months after treatment.

During the 10 days following the first treatment dose the hamster usually lost about 5 per cent of its weight. Therafter, those hamsters showing complete regression of the tumor rapidly gained weight like untreated normal hamsters. Sex did not seem to play any significant role in responding to treatment. Primary and transplantable adenovirus tumors are equally sensitive to the treatment.

In parallel studies, similar tumors were treated with saline, meat infusion broth, peanut oil, heated or autoclaved clam extract, and proteolytic enzymes such as trypsin. No therapeutic effect was observed.

The clam extract also showed local therapeutic effect against mouse melanoma which is, so far, not known to be affected by any of the anticancer drugs. Six S91 melanomas (approximately 1.0 x 1.0 cm in size) in CDF_1 mice were supplied by the courtesy of Dr. R. C. Hoye of the Cancer Institute, and four of them were given the same treatment as the hamsters received while the other two were not treated for controls. Two of the four treated mice showed complete regression within 14 days and remained normal to date (40 days after first treatment). The other two treated tumors grew again after partial regression. The two untreated tumors grew into enormous size.

3. General therapeutic effect against mouse leukemia L1210. The L1210 leukemia cells were implanted subcutaneously into DBA-2 mice. Treatment started 24 hours after implantation, consisting of 7 daily injections of the suspension of the clam extract. The injections were given subcutaneously but at an area distant from the site of implantation of the leukemia cells.

Several lots of the clam extract were tested at various concentrations. Untreated but inoculated mice served as controls. The results of 3 typical experiments are summarized here. The surviving time for 20 inoculated but untreated mice was 5.6 days. Three lots of the clam extracts were tested at the concentrations of 1000 mg/kg 500 mg/kg and 250 mg/kg using 6 mice for each concentration of each lot. The average survival time of 18 mice (3 lots) for the stated concentrations was 7.7, 7.0 and 6.2 days respectively, giving a per cent increase of survival (treated/controls) as 137, 125 and 111. These results demonstrated that although our clam material tested is still in the form of crude extract, it definitely prolonged the survival time of the treated mice over the control animals with a clear-cut dose response.

Significance to Bio-Medical Research and the Program of DBS: The results of this study indicate that the clam material, either alone or in combination with some other anti-cancer drugs, may have potential general or local therapeutic value against human cancer, or, certain types of human cancer. The crude extract has been tested by the pharmacology section at the Hazelton Laboratory and was found to be only mildly toxic.

Implication of clam extract in the development of immunity against cancer. It is generally believed that the host has the ability to destroy cancer cells under certain conditions. It is also clear that tumor viruses, after gaining entrance into the host, can create the formation of the so called "T" antigen which in turn immunizes the host against subsequent implantation of homologous tumor cells. Preliminary data in our experiments indicated that paolins by denaturing the L1210 leukemia cells *in vitro* might thereby change them into immunizing agents. They might also affect the virus-host cell inaction, helping the development of immunity. Work along these lines is in progress.

Proposed Course of Project: It is proposed that these studies be continued as follows: (1) a survey be carried out to find out whether the antitumor factor or paolin III is located in a certain organ of the clam or some other mollusks would offer better material; (2) further purification and concentration be continued by contract (Melpar) and by our laboratory in order to prepare sufficient quantity of a reasonably purified product; (3) further therapeutic and prophylactic experiments with the partially purified product be carried out against leukemia and/or other solid tumors in mice, hamsters, or other animals.

Honors and Awards: None

Publications:

Li, C. P., Prescott, B., Eddy, B. E., Caldes, G., Green, W. R., Martino, E. C., Young, A. M.: Antiviral activity of paolins from clams. Ann. New York Acad. Sci., 1965, 130: Art. 1, 374-382.

Holtz, A., Borman, G., and Li, C. P.: Hydrocephalus in mice infected with polyoma virus. Proc. Soc. Exp. Biol. & Med., 1966, 121: 1196-1200.

Serial No. 75
1. Virology & Rickettsiology
2. Basic Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on arthropod-borne viruses (arboviruses)

Previous Serial Number: 75 and 76

Principal Investigator: Alexis Shelokov, M.D.

Other Investigators: Nicola M. Tauraso, M.D.

Cooperating Units: Laboratory of Biophysics and Biochemistry, DBS
Yale Arbovirus Unit, (New Haven)

Man Years:
Total: 3.1
Professional: 1.2
Other: 1.9

Project Description:

Objectives: (1) To maintain a laboratory facility with competency in the techniques of animal work, cell culture and serology for the study of arboviruses: (2) to maintain a reference collection of arboviruses, including those of importance as vaccine candidates; and (3) to conduct studies on select arboviruses, especially those of importance to public health of the U.S. and the hemisphere as potential vaccine candidates.

Major Findings: The first objective has been achieved: the arbovirus facility now has the professional and technical competency capable of performing studies on the arboviruses, utilizing the techniques of animal inoculation, cell culture and serology. The serology laboratory regularly performs complement fixation tests utilizing the precise and sensitive "50% endpoint" measurement of complement; this procedure has been modified for use in the micro-titer equipment (this facility, now part of a larger Immunoserology Unit, also conducts COFAL tests for the avian leukosis viruses in collaboration with the Avian Leukosis Unit - for details see 2 other LVR project reports - Nos. 72 and 73. The techniques of hemagglutination and hemagglutination-inhibition are now routinely employed.

The working collection of viral and antibody reagents, summarized in the table below, has expanded considerably since the last annual report. This expanded collection is a result of the establishment of Immunoserology Unit which is developing a working competency to utilize other immunoserological methods, such as agar gel -diffusion, immunoelectrophoresis and indirect hemagglutination.

During this year studies previously reported in another report (No. 76) were continued with an emphasis being placed on the vesicular stomatitis viruses (VSV). Adult mice were immunized with a virus strain representing each of the three types (namely, Indiana, New Jersey and Cocal). After receiving two inoculations of formalinized vaccine and one inoculation of live virus, the mice were challenged with live VSV by the intracerebral route. Another group was challenged after receiving a total of four immunizing doses (two formalinized and two live virus injections). Our results indicate that significant protection develops only to the homologous virus.

Comparative electron microscopic studies on the morphology of several viruses have been initiated in collaboration with Dr. K. O. Smith, LBB. A most dramatic result was the visual demonstration that the "arbovirus" recently isolated from ticks and from patients with CNS infection (different from the classical Russian Spring-Summer Encephalitis) in reality is a typical myxovirus. The agent, described in the literature by the foremost Soviet workers, as tick-borne virus under the name of Kemerovo, was identified by J. Casals of Yale Arbovirus Research Unit and ourselves as a strain of Newcastle Disease virus. This has resulted in an over-all re-evaluation of their data by the Moscow workers. As part of this study certain select myxoviruses and other tick viruses, isolated in Siberia and Czechoslovakia and believed to be related to Kemerovo (Tribec, Lip-91 and KV-R10), were also studied by EM, in tissue culture, animals and eggs.

As an initial phase of the comparative study on the electron microscopic morphology of sandfly-related viruses, the equine prototype of vesicular stomatitis virus, Indiana type and our arthropod prototype, BT-78 (the first VSV strain ever found in arthropods which we isolated Panamanian sandflies) were photographed and identified as typical representatives of this remarkable virus group - mammalian viruses, which may cause human disease, but the morphology of which is typically that of certain insect and plant viruses.

Significance to Bio-Medical Research and the Program of the Division: Many of the continental states, as well as Hawaii, Alaska and Puerto Rico, possess the ecological prerequisites of potential vectors and reservoirs necessary for initiation of arbovirus epidemics. This has, in fact, happened in the recent U.S. outbreaks of dengue, St. Louis encephalitis, eastern and western equine encephalitis. Vaccines against these and other arboviruses of importance to the world - and the U.S. personnel and travelers abroad - are being developed. Some may soon come within the purview of the Divison of Biologics Standards.

Proposed Course of the Project: Future work will include application of some of the more precise immunological tests (e.g., fluorescent-antibody, agar-gel diffusion, etc.) and of electron microscopy to the study of arboviruses.

This laboratory offers an unusual combination of competency, facilities and interest in regard to arboviruses and avian leukosis viruses, which to our knowledge is unique in the world. This places us in a position to undertake a

comprehensive re-evaluation of the standard yellow fever vaccine known to be contaminated with the RIF agent and to attempt development of a RIF-free attenuated yellow fever virus seed. The project is now being set up as a coordinated effort involving several intramural components of DBS.

Honors and Awards: None

Publications: See below.

Shelokov, A.: Arthropod-borne viruses: the versatile parasites. In First Annual Symposium on Applied Virology edited by Murray Sanders and Edwin H. Lennette. Industrial Medicine and Surgery, Olympic Press, 1965, pp. 277-291.

Peralta, P. H. and Shelokov, A.: Isolation and characterization of arboviruses from Almirante, Republic of Panama. Am. J. Trop. Med. & Hyg., May, 1966.

Serial No. 75

CURRENT STATUS OF THE NEWLY ESTABLISHED DBS ARBOVIRUS COLLECTION

Agents	Number of Virus types	Number of Virus strains	Number of Reagents Prepared MP 1/	WP 2/	VP 3/	Ag 4/	Ab 5/
ADENOVIRUS	8	8	0	0	0	2	7
ARBOVIRUS GROUPS							
A	13	18	14	13	13	13	6
B	19	27	19	17	19	18	19
C	6	6	5	4	4	4	2
Bunya-6/	11	16	15	15	15	15	15
Calif-7/	3	4	3	3	3	3	3
Chang-8/	3	10	9	9	9	9	7
Junin	3	7	6	6	6	6	6
SFV 9/	4	8	7	7	6	4	4
VSV 10/	3	8	8	8	8	8	8
Misc.	8	9	7	6	6	6	4
Herpesvirus	2	2	0	0	0	1	1
Mycoplasma	9	11	4	4	2	3	9
Myxovirus							
Avian leukosis	4	4	0	0	0	0	0
Parainfluenza	5	5	0	0	0	1	6
Mumps	1	1	0	0	0	1	1
NDV	1	5	1	1	1	1	4
Rabies	1	6	1	1	1	1	2
Resp. Syncytial	1	2	0	0	0	1	1
Papovavirus	3	3	0	0	0	3	3
Picornavirus							
ECHO	1	1	1	1	0	0	0
EMC 11/	2	2	2	1	0	0	0
PLT Group	5	5	2	0	0	2	0
Reovirus	3	5	2	2	0	1	4
Misc. agents 12/							
Murine viruses	11/5	11/5	0	0	0	9/5	9/5
Simian viruses	2/0	2/0	0	0	0	1/0	2/0
Rubella	1	2	0	0	0	0	1
Rous Sarcomas (tumor lines in vivo)	1	4	0	0	0	4	4
TOTAL	126	184	106	98	93	112	122

Footnotes for table

1/ MP - Master Pool - a basic stock pool prepared from the "seed" which has been received by the laboratory.
2/ WP - Work Pool - a pool prepared from the MP and used for experimental work.
3/ VP - Vaccine Pool - a pool prepared from the WP for use in immunizing laboratory animals for the preparation of antibody reagents.
4/ Ag - Antigen - a standardized antigen reagent (prepared usually by special treatment of a VP) for serological work.
5/ Ab - Antibody - a standardized antibody reagent.

6/Bunyamwera, 7/California, 8/Changuinola, 9/Sandfly Fever Virus,

10/Vesicular Stomatitis Virus, 11/Psittacosis - lymphogranuloma-Trachoma,

12/Numerator - total number of agents; all or part may be included in one of the other major groups, : - Denominator - number of agents not included in another group; this number is used in calculating the total.

Serial No. 77
1. Virology & Rickettsiology
2. Basic Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Virological and immunological studies on a simian hemorrhagic fever

Previous Serial Number: Same

Principal Investigator: Nicola M. Tauraso, M.D.

Other Investigators: Alexis Shelokov, M.D.
Anton M. Allen, D.V.M.
Amos E. Palmer, D.V.M.
Robert J. Byrne, D.V.M.

Cooperating Units: Primate Quarantine Unit and Comparative Pathology Section
Laboratory Aids Branch (LAB), DRS, NIH
Laboratory of Pathology, DBS

Man Years:
Total: 0.3
Professional: 0.2
Other: 0.1

Project Description:

Objectives: To study the etiology of a hemorrhagic fever which affected monkeys held at the Primate Quarantine Unit of LAB.

1. To attempt to isolate a virus from specimens obtained from sick monkeys.

2. To perform immunological studies on sera obtained from monkeys which became clinically ill and from those which did not demonstrate evidence of the disease.

3. To attempt to reproduce the disease in monkeys housed in special negative-pressure isolators by inoculation of specimens stored at -70°C for over twelve months.

Methods Employed: Utilizing the broad resources available to the arbovirus facility of LVR an attempt is being made to establish the etiology of a simian hemorrhagic fever. The techniques of animal and egg inoculations and of cell culture are employed in an effort to isolate an infectious agent from specimens obtained from sick, dying monkeys.

The complement-fixation (CF) test is employed initially to examine the monkey sera for the presence of antibody not only to antigens prepared from the tissues of dying NIH monkeys but also to standard antigens in the arbovirus collection. Antigens prepared from sick monkeys are examined by CF tests with standard antibody reagents also in the arbovirus collection. If, and when, a candidate agent has been isolated, neutralization tests will be performed either in cell culture or in a laboratory animal system. Attempts are being made to determine the presence of hemagglutinin in the tissues of both monkeys and inoculated laboratory animals.

Major Findings: All attempts at isolating an agent in small laboratory animals, embryonated eggs or tissue culture have been negative. Utilizing a special negative-pressure isolator capable of housing two monkeys, the disease has been reproduced in rhesus monkeys by inoculation of specimens which have remained frozen at -70°C for 14 months. A kidney removed from a monkey dying from the disease was trypsinized and established in tissue culture. A viral isolate was recovered from the first and second passage of this rhesus monkey kidney cell culture. The isolate which was identified as SV40 virus failed to cause any disease when inoculated into monkeys. More intensive effort was made to isolate a bacterial or mycoplasma agent. The results were negative. Further attempts to isolate the causative agent are underway.

Significance to Bio-Medical Research and the Program of DBS: Primates are needed by the Division in carrying out the required safety tests on some vaccines. The epidemic of a febrile hemorrhagic disease which affected the rhesus monkeys held at the Primate Quarantine Unit of LAB resulted in the death of many monkeys. Several major research programs at NIH were affected by this epidemic. Because of its special mission, the Division of Biologics Standards is the largest user of rhesus monkeys and it was seriously hindered in performing one of its most important functions. Because of our facilities, the arbovirus unit of the Division became the logical group to cooperate with LAB in studying the epidemiology and etiology of this hemorrhagic disease.

Proposed Course of Project: Studies will be continued.

Honors and Awards: None

Publications: None

Serial No. 78
1. Virology & Rickettsiology
2. Basic Virology & Rickettsiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Studies on the American hemorrhagic fever viruses

Previous Serial Number: Same

Principal Investigator: Nicola M. Tauraso, M.D.

Other Investigator: Alexis Shelokov, M.D.

Cooperating Units: None

Man Years:
Total: 0.6
Professional: 0.4
Other: 0.2

Project Description:

Objectives: (1) To maintain a facility for working with the important viruses causing hemorrhagic fevers in the Western Hemisphere with emphasis on the immunological and vaccine aspects; (2) to maintain practicable cell-culture and animal-host systems and serological tools for working with this group of viruses; (3) to study the immunological relationships of these viruses using *in vitro* serological tests and *in vivo* animal protection tests.

Methods Employed: Studies were performed on three members of this group of viruses. Adult guinea pigs were immunized with live Tacaribe virus (not known to be pathogenic for any adult animal) and later were challenged with Junin virus (Argentinian hemorrhagic fever virus) to which they are normally quite susceptible. In addition to these *in vivo* challenge studies which have been completed, serological tests are being performed on the sera from the control and immunized group of animals to determine not only the amount of antibody produced but also what globulin fraction of the serum contains the antibody we are measuring. Serological studies are being performed to establish the relationship of a new isolate, Amapari virus, from Belem, Brazil, to the other members of the Junin virus group.

Major Findings: Adult guinea pigs immunized with live Tacaribe virus were able to resist challenge with a large dose of Junin virus. This immunity has lasted for at least twelve months. Adult guinea pigs immunized with live Amapari virus were unable to resist challenge with Junin virus. The Amapari-immunized animals developed very low levels of homologous complement-fixing (CF) antibodies which failed to react with heterologous Junin and Tacaribe

virus antigens. However, serological studies performed with hyperimmune antibody reagents revealed the following: by CF test Amapari virus is almost indistinguishable from other members of the group; by plaque neutralization test Amapari virus is distinct from Junin and Tacaribe viruses. We were unable to determine the relationship between Amapari and Machupo (Bolivian hemorrhagic fever virus) viruses because we lacked Machupo virus antigen and antibody reagents.

Significance to Bio-Medical Research and the Program of DBS: There is no doubt that viral hemorrhagic fevers have become a major public health concern in the Americas. So far attempts to provide a safe and adequate vaccine against the Argentinian diseases have been futile. During the most recent epidemics of Bolivian hemorrhagic fever much consideration was also given to the possibility of immunization as an eventual control measure. It would be in keeping with the mission of this Division to develop and maintain a competency able to cope with the problems of evaluating future vaccine candidates. This study indicates that immunization with an apparently non-pathogenic virus of this group may result in immunity to the epidemic virus and will serve as a foundation for this competency.

Proposed Course of Project: Future studies will endeavor to determine what globulin fraction of serum contains the antibody associated with protection in Tacaribe virus-immunized animals.

Honors and Awards: None

Publications:

Tauraso, N. M. and Shelokov, A.: Protection against Junin virus by immunization with live Tacaribe virus. Proc. Soc. Exp. Biol. & Med., 119:608-611, 1965.

Shelokov, A.: Hemorrhagic fevers in the Americas: A perspective. In "Symposium on Some Aspects of Hemorrhagic Fevers in the Americas", Am. J. Trop. Med. & Hyg., 14:790-792, 1965.

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LABORATORY OF BACTERIAL PRODUCTS (LBP)

Determination of the Potency of Poison Ivy Extracts by Clinical Studies. PH43-64-885 Orentreich Research Corporation. $23,500
Project Officer: Harold Baer, Ph.D.
Principal Investigator: Robert Auerbach, M.D. - - - - - - - - - - 16,23

Preparation of Poison Ivy Compounds. PH43-64-76 Columbia University. $122,869
Project Officer: Harold Baer, Ph.D.
Principal Investigator: Professor Charles Dawson, Ph.D. - - - - 16,23

Serological Analyses of Bordetella Pertussis Strains. PH43-66-552 Michigan Department of Health. $21,900
Project Officer: Margaret Pittman, Ph.D.
Principal Investigator: Grace Eldering, Ph.D. - - - - - - - - - 17

LABORATORY OF BIOPHYSICS AND BIOCHEMISTRY (LBB)

Analog Computer Data. PH43-65-1052 Electronics Associates, Inc. $4,450
Project Officer: C. W. Hiatt, Ph.D.
Principal Investigator: C. W. Hiatt, Ph.D. - - - - - - - - - - - - 53

Toxicological Evaluation Reports. Professional Services Contract #246940. $2,250
Project Officer: Jerome J. Helprin, Ph.D.
Principal Investigator: W. Von Oettingen, Ph.D., M.D. - - - - - -54

LABORATORY OF BLOOD AND BLOOD PRODUCTS (LBBP)

Influence of Various Materials on Human Hemo-Antibodies. PH43-65-1046 Baylor University. $166,951
Project Officer: John N. Ashworth, Ph.D.
Principal Investigator: Sol. Haberman, Ph.D. - - - - - - - - - - 67

Plastics-Biologics Interaction. PH43-64-557 University of Texas. $261,023
Project Officer: John N. Ashworth, Ph.D.
Principal Investigator: John Autian, Ph.D. - - - - - - - - - - - 67

Standardization of Blood Collection Techniques. PH43-65-1053 John S. Sharpe Research Foundation. $24,748
Project Officer: John N. Ashworth, Ph.D.
Principal Investigator: Paul Strumia, M.D. - - - - - - - - - - - 68

To Perform Studies on Hemagglutinin of Levels of Pooled Human Plasma. PH43-64-548 Southwest Blood Banks $48,609
Project Officer: Donna Williams
Principal Investigator: Marion Rymer, Ph.D. - - - - - - - - - - -

Tests Anti-Human Horse Serum by Immuno-Electrothoresis. PH43-65-98 Cordis Corporation. $21,500
Project Officer: Dr. John N. Ashworth
Principal Investigator: Dr. John Sterner - - - - - - - - - - - - -

LABORATORY OF CONTROL ACTIVITIES (LCA)
Acquire and Maintian Coral Snakes for the Extraction of Venom. PH43-65-87 Miami Serpentrarium Laboratories, Inc. $35,400.
Project Officer: Edward B. Seligmann, Ph.D.
Principal Investigator: Mr. C. E. Amory - - - - - - - - - - - - - 89

LABORATORY OF VIROLOGY AND RICKETTSIOLOGY (LVR)
Prepare, Purify, Test and Furnish Clam Extraction. PH43-66-35 Melpar, Inc. $49,100
Project Officers: Alexis Shelokov, M.D.
C. P. Li, M.D.
Principal Investigator: Mr. C. E. Amory - - - - - - - - - - - - -

SCIENTIFIC COMMUNICATIONS OFFICE (OSC)
Indexing of the WHO Working Documents from the Expert Committee on Biological Standardization. Professional Services Contract #808043. $1,200
Project Officer: A. K. Reich
Principal Investigator: Virginia Zaratzian, Ph.D. - - - - - - - - 2

BIOMETRICS SECTION
To Provide Services in the Field of Methodology Statistics, PH43-65-81 Booz-Allen Applied Research, Inc. $46,586
Project Officer: Clifford J. Maloney, Ph.D.
Principal Investigator: Mr. John Reardon - - - - - - - - - - - - - 4

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PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH
DIVISION OF COMPUTER RESEARCH AND TECHNOLOGY
Report of Program Activities
July 1, 1965, through June 30, 1966

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ANNUAL REPORT

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PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH
DIVISION OF COMPUTER RESEARCH AND TECHNOLOGY
Report of Program Activities
July 1, 1965, through June 30, 1966

This first Annual Report of the Division of Computer Research and Technology is written in remembrance of Mr. James A. King, late Associate Chief and Acting Chief of the Division. Jim King passed away on December 29, 1965, just a short time after the formal establishment of the Division. His devoted care to each immediate concern of the Division and to its future role within the NIH community, his patient skill in converting difficult problems to useful courses of action, and above all, his complete integrity of character will be affectionately remembered by all who knew him.

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A. Objectives

The purposes of the Division are: to pursue independent as well as collaborative research in mathematics, mathematical statistics and computer programming; to design, develop and implement computer-based data processing systems and special-purpose equipment to meet research and management needs at NIH; and to provide a broad span of programming and computational services to support these needs.

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B. Current Programs

During this, the first full year of operation of the Division, major attention has been devoted to (1) the recruitment of experienced mathematicians, mathematical statisticians, electronic engineers, systems analysts, and programmers, and (2) the initiation of mathematical research projects and the design of computer-based information processing systems urgently needed in a number of NIH program areas.

The research efforts described in individual project reports include mathematical and statistical studies of (1) sequencing of nucleic acids and proteins, (2) mechanisms of flow of bodily fluids, (3) automated biochemical processes, and (4) blood chemistry tests of normal controls and Clinical Center patients.

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Computer systems are currently being designed, revised or tested in the areas of cardiology, neurology, cancer chemotherapy, Clinical Center activities, and central management functions.

The Division now has approximately 240 employees. In addition to the Office of the Chief, four branches are authorized: Computation and Data Processing, Mathematics and Programming Research, Data Systems Analysis, and Computer Equipment. Of these, the Computation and Data Processing and the Data Systems Analysis Branches are in operation. The former, transferred from the Division of Research Services, comprises some 200 computer programmers, analysts, and operating staff. The latter, activated

during the reporting period, now numbers approximately 25 employees.

C. Summary of program progress and accomplishments

1. Conversion to new central computer system

Since July 1, 1965, following selection of the IBM 360 computer system to replace the Honeywell 800 computers in the NIH central computing facility, the Division has been working with IBM programmers under contract to convert current Honeywell systems to the 360. Major effort during FY 1966 was devoted to (a) conversion and testing of mathematical and mathematical-statistical FORTRAN programs as well as programs for the processing of administrative management information and (b) specification, following a four-months study, of an improved computer processing system for research grants administration. Progress in (a) has been slower than anticipated, due chiefly to late delivery of IBM software for the 360 computer series, but also in part to the lack of completely adequate documentation for existing computer programs. Modifications and improvements to the research grants system have been defined and approved. Conversion testing and operational use of 360 mathematical and administrative computer programs will probably not be completed until September, 1966. Prior to that time conversion of epidemiological, information retrieval, research grants, and related computer systems will have commenced.

A continuous activity of training Division and other NIH programming personnel in 360 systems has been undertaken. Since April 1, 1966, all new programs are being written for the 360 series. A 360 Model 40 was installed on April 1, 1966, and upgraded to a Model 50 during May. A Model 30 was installed in May for emulation and conversion of Mental Health Clearinghouse and Cancer Chemotherapy information processing systems now on contracts.

2. Installation of hybrid computer

During this year an advanced hybrid computer built to unusually stringent speed and accuracy specifications was installed by the Division in the Clinical Center, for immediate application to cardiologic and lung function research in cooperation with Dr. Donald L. Fry and his associates in the Cardiology Branch of the Heart Institute. A group of Division employees is collaborating with Heart Institute and other scientists on a continuous basis to assure most effective use of the hybrid system. The group currently includes two electronic engineers, a mathematician, two computer programmers, and a computer operator. The hybrid computer completed its final acceptance testing during the last quarter of FY 1966.

3. Computer programs for heart surgery

In collaboration with Dr. Andrew G. Morrow and other Heart Institute scientists, Division staff are developing a specialized library of computer programs for the processing of continuous information recorded from patients during heart surgery. These programs will compute many parameters of heart function for study by Division and Heart Institute research scientists to

gauge possible effects of surgery. Although the library is being written initially for operation on the Honeywell system, it will shortly be converted to the hybrid computer and may eventually form the basis for real-time computation in the heart surgical suite.

A start has been made on the mathematical definition of collaborative research interests of the Division in areas of aortic flow, turbulent blood flow, and ventricular contraction. At this point, however, progress has not been sufficient to warrant detailed description in individual research projects.

4. Support of Clinical Laboratory Automation

During FY 1966, the Division devoted approximately $400,000 to the support of on-line automation of laboratory procedures in the Clinical Pathology Department of the Clinical Center. Most of these funds were used to purchase existing computer equipment (part of the Control Data Corporation 3200 System in Clinical Pathology) and related laboratory equipment, and to finance real-time programming contracts. In addition, the Division is funding the further development of information storage and retrieval programs for laboratory data, some to be operational on the CDC 3200, others for back-up and statistical research studies on the central 360 facility. The total effort has permitted increases in the number of completely on-line laboratory tests during this fiscal year from six to twelve, and of partially automated tests from 18 to 50. Further, new programs for the structuring of historical patient data files have made possible the beginning of an extensive statistical research program in the analysis of clinical laboratory information (see Individual Project Report Number 4 on this subject).

5. Computer systems design

Several major systems design projects initiated during FY 1966 are noted briefly here and described more fully in the report of the Data Systems Analysis Branch.

a. Cancer Chemotherapy

A joint team of systems analysts and programmers from the Cancer Institute and the Division began redesign and conversion of the cancer chemotherapy data processing system, now being handled under contract on IBM 1401/1410 computers, to a 360 Model 30 in the NIH central facility. The project provides for (a) reprogramming a major portion of the present system to achieve greater operating efficiency, and (b) emulation of the remainder.

b. Clinical Center patient care

Systems analysis and preliminary design of suitable data processing methods was begun in several patient care activities of the Clinical Center, including pharmacy and sterile supply, admissions, and routine medical records functions. Specifications were completed for a contract to

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supplement the Division's efforts in the design of computer systems for the pharmacy and the scheduling of inpatient and outpatient services, and to assist in the development of a general on-line system to encompass all relevant patient data.

Development of an information-handling system was begun in cooperation with Cancer Institute clinicians and the Clinical Pathology Department of the Clinical Center. Its program is to facilitate the coded recording and eventual machine processing of medical information on leukemic patients under drug therapy.

c. Central management functions

In cooperation with the Management Policy Branch, OAM, preliminary studies were begun in the personnel, supply, and financial management areas, looking toward an integrated computer system to facilitate the production of timely, useful reports to administrative management.

d. Mental Health Clearinghouse

Plans were initiated for the formation of a joint Division/NIMH systems team to study the functions of this document storage and retrieval activity. The ultimate objective is to redesign and convert the activity to the central 360 computer facility. Meanwhile, agreement has been reached with Mental Health to emulate the current system on the 360 Model 30.

6. Neurophysiological computing requirements

During the latter half of FY 1966, a small systems engineering group from the Division in cooperation with Dr. Maitland Baldwin, Chief of the Surgical Neurology Branch, NINDB; Dr. Robert M. Farrier, Associate Director, Clinical Center; and the Biomedical Engineering and Instrumentation Branch, DRS, studied current requirements and potential applications of an on-line, real-time computing facility to serve the neurosurgical suite and associated primate laboratories. The study report, now under review, includes specifications for a suitable experimental computer system. Continuing examination of literature and data related to neurosurgery will determine plans for immediate off-line computer analysis.

7. Remote terminals

During the year a beginning was made in the experimental use of remote terminals linked to time-sharing computer systems. By June 30, 1966, some half-dozen teletypewriter consoles, including one in the Division, will be in operation in various NIH laboratories. They will use relatively limited mathematical programming systems to transmit data and programs to commercial computers outside NIH. The Division has arranged for each user to maintain a log of time used, types of programs and data transmitted, and problems encountered. The users will meet regularly as a group to discuss their experiences with these terminals.

8. DCRT satellite library

Agreement has been reached with DRS on the establishment of a satellite library in DCRT. The library will be the primary NIH resource for books and other publications in the computer field and will conduct experiments in automated indexing and document retrieval.

D. Problems

Management

The Division's programs were seriously hampered during its first year of operation by inadequate top management resources. This problem should be overcome in FY 1967 through the filling of key positions in the Office of the Chief and the various branches.

Recruitment

A major continuing problem is the difficulty in recruiting urgently needed qualified mathematicians, mathematical statisticians, computer systems analysts, electronic engineers, and programmers. The problem is essentially a combination of two factors: a scarce market and cumbersome governmental hiring procedures.

Computer Conversion

Late delivery of adequate IBM software has caused delays in the conversion to the 360 system. The delays have placed unexpected burdens on the customers whose programs are affected and required major and time-consuming adjustments by the Division's staff.

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July 1, 1965, through June 30, 1966

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH
DIVISION OF COMPUTER RESEARCH AND TECHNOLOGY

Summary of Branch Activities

1. DCRT-2
Serial Number

2. COMPUTATION AND DATA PROCESSING BRANCH

3. H. J. Juenemann
Acting Branch Chief

I. SUMMARY

The Computation and Data Processing Branch has concentrated much time and effort during Fiscal Year 1966 to improving its capability as a central ADP facility for the National Institutes of Health. Extensive training and conversion activities were undertaken to prepare for the installation and operation of an IBM 360 computer system which will replace the existing Honeywell computers. These efforts are being accomplished with little or no interruption to the services offered by the Branch.

II. BRANCH PROGRAMS

A. Objectives

To provide centralized computation and data processing services, using electronic digital computers, remote consoles, electric accounting machines, oscillogram scanners, plotters, and other data processing equipment.

To provide mathematical, statistical, and programming consultation service in support of the research and administrative programs of NIH.

To develop software and problem-oriented computer languages to permit more effective and more efficient use of the resources of the Branch.

B. Current Programs

The major Branch efforts currently under way are directed toward reprogramming the present systems to make them compatible with the IBM System 360. The majority of the Branch staff, both programmers and machine operators, are receiving training in the new system. Although this has cut sharply into available productive staff time, CDPB continues to provide uninterrupted ADP service on a fee-for-services basis at costs which compare most favorably with current commercial rates. Presently, 546 research support and administrative projects are receiving the ADP, mathematical, and programming services of the Branch.

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C. Program Progress and Accomplishments

1. Acquisition of New Computer Systems

Fiscal Year 1966 was one of conversion and growth for the Computation and Data Processing Branch. The selection of an IBM 360 computer during the closing months of FY 1965 had a profound effect on all areas of the Branch, which had operated Honeywell computers since 1960. In preparation for the April 1966 delivery of the first phase of the 360 system, CDPB's mathematicians, programmers, operators and schedulers, as well as open shop programmers, received formal classroom and on-the-job training in the assembly languages and operating systems of the 360 computer. CDPB's library of computer programs, written for the Honeywell system, was converted for use on the 360. Adaptations were required in the card punch equipment to afford compatibility with the new computer. An additional 2,721 square feet of office space in Building 12 was converted to accommodate the new system.

In addition to the IBM 360 model 50, which was acquired for general data processing and scientific computation, an IBM 360 model 30 was acquired, primarily to enable the Branch to process the National Clearinghouse for Mental Health Information retrieval system and the Cancer Chemotherapy data processing system. Both systems have been processed under outside contracts in the past. The Branch subscribed to a remote computer service which provides semi-conversational mode access to a computer located at CEIR by means of a console located in the Branch. The console consists of a tele-typewriter and Data-Phone linked to the computer, which is commercially operated and located in Arlington, Virginia. In addition to rapid access for small jobs, this equipment will provide the Branch with valuable experience in the operation, management and use of remote consoles in preparation for their installation in connection with the NIH 360.

2. Equipment Purchases

The purchase by the Branch of a significant amount of previously rented IBM tabulating and computer equipment resulted in a decrease in monthly charges for machine rental. The savings were passed on to the customers in the form of reduced hourly rates for many EAM operations.

3. Training

Approximately 155 laboratory scientists and other interested NIH personnel were taught computer technology, programming, operations techniques, and computer applications in a formal training course conducted by the Branch. In addition, CDPB was represented by three employees on the faculty of the Foundation for Advanced Education in the Sciences, the Graduate Program at NIH.

4. Significant Projects

The following projects are singled out as significant examples of CDPB's

The Computation and Data Processing Branch is currently creating a central data base to be used for statistical tabulations. These include: family census tables (from 1830-1964), life tables, population increments and decrements, family size tables, mating patterns, and inbreeding coefficient calculations.

f. Biometric Study of Steelworkers

A file was created for the Biometry Branch, National Cancer Institute, containing vital statistics and work histories for 59,072 men employed by the steel industry in Allegheny County during the years 1953-1961. From this file, a number of listings and tables have been generated as adjuncts to the Biometry Branch's investigation into the possible relationships between the amount of "person years risk" incurred during various steel plant jobs and the causes of deaths occurring during the study period. Exposure in the coke ovens is of particular interest, and a number of tables have focused on jobs in these areas.

g. Framingham Project

Editing of data is near completion for the Framingham project. This is a study of 5,209 individuals who have been and are being intensively tested and interviewed in Framingham, Massachusetts, with particular emphasis on heart diseases. More than 700 variables, such as age, sex, and nationality are being considered. The results will be tabularized and subsequently analyzed by a computer.

5. Publication

Ellison, J., Kiefer, J.: The prediction paradox again. Mind Vol. LXXIV, N. S. pp. 426-427, July 1965.

D. Problems

1. Recruitment

The difficulties encountered in replacing operations personnel lost through normal attrition is the most serious unresolved problem. These difficulities include inadequate Civil Service Commission registers of eligible applicants from which to select EAM and computer operators. In addition, applicants who are located by the Branch directly, and those who may be selected by the Branch, have encountered unnecessary difficulties and delays in their personnel processing. As a result, a high percentage have declined the offered positions before they could be entered on duty.

2. Storage Space

Inadequate storage space for ADP supplies has posed a continuing problem. However, if the tentative assignment of 1,000 square feet of space in the basement of Building 12A is confirmed, and if none of the presently occupied storage space must be relinquished, the problem will be much less acute.

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contributions to the NIH research community during the past year:

a. Pathology Information System

Work continued on an expansion of the Pathology Information processing system. The system now provides for multiple displays of patient records which can be retrieved in accordance with a logically structured, multicomponent search query related to the diagnostic, patient identifying, and vital statistics data within a single record. The continuing experimental evaluation indicates that the nomenclature (Systematized Nomenclature of Pathology) is complete enough to provide automatic storage (automatic coding) and retrieval of most pathology data.

b. Internal Indexing

A preliminary study of the preparation of indexes for books and articles (internal indexes) has been made during the past year. Internal indexing is characterized by the fact that relatively detailed coverage is desired compared to the level sought in external indexing. This goal may be achieved by providing a dictionary of common words in the computer memory and treating all other words as candidate index entries and performing a final manual screening of the computer output. In addition, the context of sentences being processed is preserved.

c. Computer Analysis of Lung Models

Various lung models have been derived in the Heart Institute. They have been three-dimensional representations in which transpulmonary pressure, respiratory flow, and lung volume have been related. A number of computer programs have been written to develop and analyze these models with experimental data obtained in the lungs of animals and normal and diseased human subjects.

d. Nuclear Resonance Abstract System

A Nuclear Magnetic Resonance abstract literature retrieval system was developed for NIAMD. The system searches tape files with authors and/or pre-set subject codes as grouped or engrouped keys; it also contains an update routine. The system is also being utilized by the Food and Drug Administration.

e. Brandywine Study

A population consisting of 5,000 people of mixed Caucasian, Indian and Negro ancestry, located south of Washington, D. C., is the subject of an NIH research project. NIH has chosen to study this group, known as the Brandywine population, because of the high rate of intermarriage during the past 250 years (the group marries, for the most part, within only 14 surnames). Thus, during the summers of 1957, 1958, and 1960, field examinations (physical, dental, laboratory and blood) were conducted on this population. Information has also been obtained on their ancestors.

E. Program Plans

1. Expansion of Service

The services of the Computation and Data Processing Branch are being expanded to provide more powerful and more sophisticated tools for the processing of the mathematical, statistical, and data reduction requirements of NIH.

A larger central computer facility, remote consoles for access to the computer, and executive operating systems which permit multi-processing of computer programs and time sharing of the computer facilities by a large number of independent users, are being planned and are expected to be implemented within the next fiscal year.

2. CDPB Standards Manual

A printed CDPB standards manual will be released early in Fiscal Year 1967 for use by Branch employees and customers. Its primary purpose is to disseminate information on policy and procedural changes associated with the conversion to a new computer system. The manual provides a convenient collection of CDPB administrative, programming, and operating standards and procedures. It is expected to result in more effective computer management.

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PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH
DIVISION OF COMPUTER RESEARCH AND TECHNOLOGY

Summary of Branch Activities

1. DCRT-3
Serial Number

2. DATA SYSTEMS ANALYSIS BRANCH

3. Dr. E. K. Harris
Acting Branch Chief

I. SUMMARY

During the reporting period the Branch installed a hybrid computing system in the Clinical Center and developed much of the software required for the system; began development of automated systems for patient care and related activities in the Clinical Center; completed a project plan and schedule for converting the Cancer Chemotherapy data system to the IBM 360; began planning for the design of a cancer patient information system; completed a survey on the current use of computers in neurological research and developed a request for proposals for a computer system for use in neurosurgery; and initiated a study of NIH supply functions with a view to their eventual integration in a single supply and equipment system.

II. CURRENT BRANCH PROGRAMS

A. Objectives

To develop and operate a hybrid analog/digital computing system and to conduct research directed toward fostering the application of hybrid systems to biomedical research.

To automate and integrate Clinical Center patient care activities and the flow of patient information.

To develop and implement biomedical information systems in support of research on diseases.

To develop and foster the use of on-line, real-time computing in the NIH clinical research environment.

To design, develop, and implement computer supported integrated management systems for NIH.

B. Progress of Current Programs

1. Hybrid Computer Developments

a. The hybrid analog/digital computing system was installed in the Clinical Center during the reporting period. Work was begun on the

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definition and writing of a series of connected computer programs to retrieve and analyze in digital form continuous recordings of ventricular pressure and flow, atrial pressure, aortic pressure and flow, and the electrocardiogram measured before, during, and following heart surgery. These programs include the Fourier smoothing and recomposition of the variables involved, the automatic determination of the systolic ejection phase, and the computation of heart power, stroke volume, and stroke work at consecutive points in time during the contractile period. Although developed for operation on the Honeywell computers, it is expected that this program library will be applied to the hybrid system.

b. A magnetic tape coding and searching system has been designed and assembled. Plans are to use these devices in conjunction with an analog to digital conversion program in order to provide computer controlled searching of analog tapes.

c. Communication has been established with a staff member at the University of Maryland concerning the use of digital-analog simulators.

d. Preliminary analysis of the lung mechanics model has been carried out to determine the complement of analog equipment required for solution. A purchase order has been issued for this equipment.

2. Automation of Clinical Center Activities

a. Pharmacy: Automation of Internal Operations

This project involves analysis and documentation of the present operating procedures of the Clinical Center Pharmacy and the design and implementation of new, moderately sophisticated, off-line computer systems for controlling pharmaceuticals and sterile supplies. Included in the project is the development of an entirely new accounting system for allocating the costs of consumption of these supplies.

Status:

Initial documentation and preliminary analysis of procedures for the ordering, receipt, and issue of drugs from the pharmacy has been completed, including procedures for issuing prescriptions and for prepackaging and bulk compounding drugs. In addition, preliminary documentation has been accomplished for nursing unit drug control and administration, investigational drug control, and I.V. additive control and administration.

b. Medical Record: Automation of Reports Derived from Statistics on Patient Populations and Activities

This project involves analysis of inpatient and outpatient admissions, transfers, and discharges, and the design of an elementary, off-line, computerized system for producing approximately eight

periodic reports now produced manually by the Admissions and Medical Record Departments. The utility of the various functions involved will be analyzed, with a view to relocating, consolidating, or eliminating functions as appropriate.

Status:

Work was begun only recently. The design phase is progressing smoothly.

c. Indicative Interface #1: Clinical Pathology Laboratory

The ultimate objective of this project is to provide real-time interface linkages between peripheral computer facilities, such as that of the Clinical Pathology Department, and a central computer facility. The purpose of these linkages would be to transmit indicative, key, and index information from the ancillary operation to a central data base, and to provide a path back to the ancillary operation for detail information requests triggered at the central facility by various research inquiries. In this project's initial effort, analysts of the Branch are working with clinicians of the Cancer Institute and the Clinical Pathology Department on the design and implementation of a subsystem to mechanize processing of clinical observations on drug therapy in leukemia.

Status:

The project is in the planning stage.

d. Contract to supplement DCRT systems effort in the Clinical Center

Branch analysts, in cooperation with Clinical Center staff, performed the preliminary analysis and developed the specifications for a request for proposals for a contract to supplement the Division's efforts in the automation of Clinical Center activities.

3. Cancer Chemotherapy Conversion Project

Staff of the Branch and the Cancer Institute are collaborating in the conversion and moderate sophistication of two IBM 1401 systems (Drug Evaluation and Endocrine Evaluation) of the Cancer Chemotherapy program, now operated under contract by Documentation Incorporated, to operate on the IBM 360 Model 30 in the NIH central facility. The effort includes reprogramming of three major drug evaluation programs, modification of five ancillary programs, validation of thirty-five programs for operation in the emulation mode, and development of systems documentation.

Status:

A project plan and schedule for implementation has been completed using the PERT technique. Resources required for project implemen-

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tation have been acquired, and programmer training is in the final phase. Acquisition of current program documentation and preparation of program modification specifications are in process. The reprogramming effort is in the problem analysis phase. The target date for project completion is December 31, 1966.

4. Cancer Patient Information Systems Design

A beginning has been made in the design of a subsystem within a major module of the projected Cancer Information System, specifically related to cancer treatment, with immediate focus on clinical trials information processing. The initial effort entails intra-Branch collaboration on the analysis and design of the subsystem for implementation within the Clinical Center.

Status:

The project is in the planning phase.

5. Neurosurgical Computing System

A project was begun during the last quarter of FY 1966 to provide an on-line, real-time computing system for surgical neurology. This system would provide a unique opportunity to exploit recently developed mathematical methods and to explore new mathematical and physiological concepts related to the reception and transmission of information in the brain. When used during neurosurgical research procedures, the computer would provide timely information to the surgeon on matters of immediate importance to him, such as the proper placement of electrodes, the usefulness of averaging procedures in maximizing signal to noise ratios, and the correlative relationships among many neuronal, physiological, and behavioral variables being measured concurrently and continuously.

Status:

a. A brief survey of the state of the computer art in a neurological environment was completed. It indicates that NIH must make a concentrated effort to stay abreast of recent developments elsewhere.

b. A request for proposals for a computer system has been prepared, containing minimum requirements the system must meet to be considered responsive. The requirements were derived by studying the needs of current and anticipated projects.

6. Automation of Supply Functions

An analysis of Supply Management Branch functions has been undertaken, with the objective of relating and integrating in one system all supply equipment functions at NIH. It is hoped that eventually all processes in supply touching upon other areas, such as fiscal accounting, could be automatically related and integrated through the use of

central files processed by the central computer facility of the Division. In achieving these objectives, full advantage will be taken of exception reporting, simulation, and other techniques to provide management with the data necessary for timely and more accurate decisions.

Status:

The study is in the data gathering phase.

C. Problems

1. Recruitment

The scarcity of qualified systems analysts poses a major problem for all program areas of the Branch.

2. CCNSC Conversion Project

The Branch is having difficulty in acquiring from the contractor valid program source and object decks and sufficient valid program documentation to serve as a basis for reprogramming the Cancer Chemotherapy project. The lack of documentation is being overcome through the tedious, time-consuming process of developing program logic flow charts based on detailed analysis of available program listings.

D. Program Plans

1. Hybrid Computer

During FY 1967 pending research projects will be carried through in cardiac lung mechanics. These will include simulation and optimization of the heart and lung mechanics models, with appropriate modification of the models to obtain information of greater physiological significance. Support will be given to current studies of arterial blood flow, shearing stress analysis, spectral density studies of turbulent blood flow, and tests of biochemical process automation.

2. Automation of Clinical Center Activities

Plans for Clinical Center automation in FY 1967 include the following:

a. Completion of automated systems for the Pharmacy;

b. Launching of a major operations research effort to study and develop mathematical, computer-based techniques for the scheduling of clinical services;

c. Attainment of full mechanization of clinical trials on drug therapy in leukemia.

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3. Cancer Drug and Endocrine Evaluation

A follow-on project is planned for redesign and reprogramming of Drug and Endocrine Evaluation systems to provide marked systems improvements, increased scope, and modularity with respect to other existing or projected subsystems supporting related operational cancer research functions.

4. Cancer Pharmacology

The Branch will begin a new project to analyze the cancer pharmacological functions with a view to improving them through ADP systems design.

5. Neurosurgery computer

Plans in this area for the current year include:

a. Acquisition of a computer system for neurosurgery;

b. Training and orientation of interested investigators in the use of the system;

c. Development of mathematical statistical methods for effective analysis of neurological data;

d. Study of the possible use of display devices and the computer to record electroencephalographs and to permit greater and more selective use of electroencephalographic data;

e. Possible development of a conversational type language for display of data, curves, computations, etc., during neurological experiments; and

f. Exploration into the potential uses of on-line, real-time techniques in heart research, intensive patient care, and servo-anesthesia.

6. Automation of management functions

Plans for the current year include systems studies of the NIH Service and Supply Fund, collaboration with the Management Policy Branch on the development of a personnel management information system, systems analysis in the areas of budget and fiscal accounting, and assistance in the development of a management information system for DCRT.

Serial No. 3.1
1. Data Systems Analysis
2. Not Applicable
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Mathematical Problems of Sequence Determination of Nucleic Acids and Proteins

Previous Serial Number: None

Principal Investigator: James E. Mosimann, Ph.D.

Other Investigators: Marvin B. Shapiro, Jay Vinton

Cooperating Units: Laboratory of Neurochemistry, NIMH

Man Years (computed for the 12 month period)
Total: .75
Professional: .5
Other: .25

Project Description:

Objectives:

(1) To provide mathematical definitions of classes of protein and nucleic acid sequences which cannot be determined using complete digest methods.

(2) To provide a probability framework for evaluating the probability that protein and nucleic acid sequences of certain classes are solvable, using partial digest methods.

(3) To provide mathematical support necessary for testing computer programs designed to reconstruct nucleic acid and protein sequences from fragment data.

Methods:

The primary mathematical tools are (1) algebra, specifically the algebra of free monoids; (2) graph theory; and (3) stochastic processes, Markov chains.

Major Findings:

There are broad classes of sequences which cannot be determined by complete digests with monobase specific enzymes. The theoretical studies clearly indicate the need for partial digest fragment data in determining sequences of nucleic acids.

Significance to Biomedical Research:

Determination of the primary structure of proteins and nucleic acids is of extreme importance in studies of the genetic code, as well as molecular bases of life.

Honors and Awards: None

Publications:

Mosimann, James E. (with M. B. Shapiro, C. R. Merril and D. F. Bradley): Reconstruction of Protein and Nucleic Acid Sequences: Ala SRNA. Science. 150: 918-920, 1965.

Mosimann, James E. (with M. B. Shapiro, C. R. Merril, D. F. Bradley and J. E. Vinton): (in press) Reconstruction of Protein and Nucleic Acid Sequences IV. The Algebra of Free Monoids and the Fragmentation Stratagem. The Bulletin of Mathematical Biophysics. 1966.

Mosimann, James E. (with C. R. Merril, M. B. Shapiro, D. F. Bradley and J. E. Vinton): Reconstruction of Protein and Nucleic Acid Sequences V. Computer-Simulated Tests of Various Tactics for Reconstructing the Sequences of Transfer Ribonucleic Acids. Biopolymers. 1966.

Serial No. 3.2
1. Data Systems Analysis
2. Not Applicable
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Automated Sequencing Laboratory

Previous Serial Number: None

Principal Investigator: Marvin B. Shapiro

Other Investigators: James E. Mosimann, Ph.D., Jay E. Vinton

Cooperating Units: Laboratory of Chemistry, NIAMD; Laboratory of Neurochemistry, NIMH.

Man Years (computed for the 12 month period)

Total:	1.5
Professional:	1.0
Other:	.5

Project Description:

Objectives:

The basic goal of the project is the automation of the entire procedure followed in a biochemist's laboratory for finding the primary structure or sequence of a protein.

Methods Employed:

A small digital computer will accept and analyze real-time data and exercise feedback control over the laboratory operations, and a large digital computer will be used to make complex decisions for optimizing the process in terms of running samples which yield a maximum amount of information. Work has begun on programming a small real-time computer, the CDC 3100, to accept data on-line from an amino acid analyzer and to control the operation of the analyzer to allow around-the-clock operation, including control of pumps and valves and automatic loading of samples.

Major Findings:

Theoretical work on the logic and mathematics of the sequencing problem has continued over the past year and has resulted in completion of a complex computer program for reconstructing sequences from sequence fragment data and in the development of a mathematical theory explaining insolvable sequences.

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Significance to Biomedical Research:

With the present computer program, sequence data can be handled faster and more accurately. This allows investigation of larger proteins than have been looked at up to now.

Proposed Course of Project:

The computer program will continue to be developed to handle all types of available protein sequence data and to tie in with the decision-making part of the automation project. The automation will be developed to connect more than one analyzer and other instruments to the computer, to produce a more complete analysis of the data, and to design and implement a system for handling the large amounts of protein data collected.

Honors and Awards: None

Publications:

Shapiro, Marvin B. (with J. E. Mosimann, C. R. Merril and D. F. Bradley): Reconstruction of Protein and Nucleic Acid Sequences: Ala SRNA. Science. 150: 918-920, 1965.

Shapiro, Marvin B. (with J. E. Mosimann, C. R. Merril, D. F. Bradley and J. E. Vinton): (in press) Reconstruction of Protein and Nucleic Acid Sequences IV. The Algebra of Free Monoids and the Fragmentation Stratagem. The Bulletin of Mathematical Biophysics. 1966.

Shapiro, Marvin B. (with J. E. Mosimann, C. R. Merril, D. F. Bradley and J. E. Vinton): Reconstruction of Protein and Nucleic Acid Sequences V. Computer-Simulated Tests of Various Tactics for Reconstructing the Sequences of Transfer Ribonucleic Acids. Biopolymers. 1966.

Serial No. 3.3
1. Data Systems Analysis
2. Not Applicable
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Basic Studies on Viscous Effects on Flow Mechanisms of Biological Fluids in Capillaries and Other Microscopic Structures

Previous Serial Number: None

Principal Investigator: Joel D. Isaacson, Ph.D.

Other Investigators: John E. Fletcher

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: .5
Professional: .5
Other: 0

Project Description:

Objectives:

Many flow phenomena in small blood capillaries and within living cells are not understood in terms of classical hydrodynamics. In particular, flows that should be laminar by standard criteria (such as Reynolds number) are not necessarily so, as observed in motion pictures of capillary blood flow. In addition, most biological fluids are markedly non-Newtonian, so that the classical Navier-Stokes system is not applicable to biological flow problems without appropriate modifications.

The objective of this study is to construct a more general viscosity-model for non-Newtonian biological fluids, so that subsequent modelling of their flow characteristics in the biological microstructure in terms of modified Navier-Stokes systems will become more meaningful.

Methods and Preliminary Findings:

A two-parameter viscosity-model is investigated in the form

$$\sigma = - \alpha v^{-\beta} \frac{dv}{dx} \qquad [1]$$

where

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σ is the viscous (shearing) stress

v is the velocity

$\frac{dv}{dx}$ is the velocity gradient

α is the first ("Newtonian") viscosity coefficient (α real and positive)

β is the second ("non-Newtonian") viscosity coefficient (β real and positive).

For one-dimensional flow, (between two infinite plates 2a distance apart), the viscous term in the Navier-Stokes equation becomes

$$\text{Viscous Term} = -\alpha \frac{1}{v^{\beta}} \frac{d^2v}{dx^2} \qquad [2]$$

and for incompressible, steady flow, the flow is described by the following second-order non-linear differential equation

$$\frac{d^2v}{dx^2} + \lambda v^{\beta} = 0 \qquad [3]$$

where $\lambda = \nabla P/\alpha$ is a parameter, and ∇P is the pressure gradient = constant. Various boundry conditions can be prescribed to complement this equation. The simplest:

$$v(a) = v(2a) = 0. \qquad [4]$$

The solution of Eq. [3] is written in the form of an elliptic integral as follows,

$$x = \int \frac{k_1 \, dv}{\sqrt{1 - v^{\beta+1}}} \qquad [5]$$

where

$$k_1 = \left[\frac{(\beta+1)}{2a^{\beta-1} \lambda^{\beta}}\right]^{\left[\frac{1}{\beta+1}\right]} \qquad [6]$$

and

$$v = k_2 v \qquad [7]$$

where

$$k_2 = \left[\frac{\beta+1}{2} a^2 \lambda\right]. \qquad [8]$$

The solution, Eq. [5], was investigated for the Newtonian case, [$\beta = 0$], and for the first non-Newtonian case, [$\beta = 1$]. Both cases proved to be consistent with standard derivations.

More complex non-Newtonian cases for $\beta > 1$ are now investigated. In particular, attention is focused on the effect of large β's on the velocity profiles, as $\underline{a}$ becomes extremely small.

No experimental work is currently being done on this project. However, collaboration is contemplated with one or more experimentalists on various aspects of microscopic flow, particularly blood flow in capillaries.

Significance to Biomedical Research:

The mathematical study of non-Newtonian viscous flow is necessary to an understanding of the passage of body fluids through very fine tubes and other microscopic structures (e.g., blood flow in small capillaries).

Proposed Course of Projects:

a. Hybrid Computer

The DCRT hybrid computer is being programmed to model the one-dimensional, steady, incompressible, logarithmic-viscous flow, arising from the case $\beta = 1$ of our theroy. It is hoped that more insight can be gained into the problem through visual results of velocity profiles as affected by change in various parameters obtained from the Calcomp plotter (the hybrid system).

b. Digital Computers

In the future a computer and recorder employing special output routines will be needed to describe visually complex time-dependent flow problems, such as the motion of red blood cells in a capillary. One such routine displays the silhouette of a red blood cell on 35mm film and can rotate it 360° at 5° intervals, as well as move it in any direction according to flow calculations.

nors and Awards: None

blications: None

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Serial No. 3.4
1. Data Systems Analysis
2. Not applicable
3. Bethesda

PHS-NIH
Individual Project Report
June 1, 1965 through June 30, 1966

Project Title: Statistical Research in Clinical Pathology

Previous Serial Number: None

Principal Investigator: Eugene K. Harris, Ph.D.

Co-Investigator: Ernest Cotlove, M.D.

Cooperating Unit: Clinical Pathology Department, Clinical Center

Man Years (computed for the 12 month period)

Total:	1.0
Professional:	.25
Others:	.75

Project Description:

Objective:

To explore, through careful application of statistical methodology, relationships inherent in large volumes of results of chemical analyses routinely performed on serial blood samples from normal volunteers and patients at the Clinical Center.

Method:

At this time, blood chemistries from 79 normal volunteers, in ten groups of seven or eight individuals per group, are stored in easily retrievable form on digital magnetic tape. These data consist of concentrations (milli-equivalents per liter) of at least 15 elements and compounds (including some enzymes) measured in each of 10-12 weekly blood samples from each subject. The total ten-group data base covers a $2\frac{1}{2}$-year period of study.

Similarly stored patient blood data are available from approximately 2500 Clinical Center admissions during a 9-month period in 1964-65. Demographic and diagnostic information on these patients is also available for related study. Serial blood data for each patient is less abundant than for normal volunteers and not regularly spaced, but does permit some time-wise analyses relative to effects of therapy or other changes of state.

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Initially, analysis of variance computer programs have been applied to the weekly concentrations from normal volunteers (79 subjects, 15 blood tests, each in duplicate, 10-12 samples per subject--a total of roughly 25,000 observations) to determine analytical variation and the significance of variation from week to week and among each group of subjects.

Major Findings:

Preliminary results indicate consistently "high," "low," and "intermediate" subjects within each group with respect to any one blood test, little meaningful variation over time, adequate to excellent agreement between duplicate analyses. Ranking methods will be used to confirm this apparent consistency within subjects and, further, to provide a crude measure of clustering among sets of blood variables.

Significance to Bio-medical Research:

These studies will (1) add substantially to existing information on equilibrium levels of blood constituents and (2) provide new knowledge on currently achievable analytical precision and on time-dependent variations due to patient differences, disease states, and effects of therapy.

Proposed Course of Project:

Continuing statistical study of normal volunteer data will isolate and quantify consistent sources of variation. Although factor analysis is not likely to be too illuminating since no subject has more than 12 successive sets of blood tests, this means of elucidating relationships between variables will be investigated.

Attention will next be centered on the patient file, using distributions of blood tests in normal volunteers to aid in isolating extreme ranges in each variable among patients. Correlations among extremes and relationships to diagnosis and to demographic variables will be examined, and relevant physiological hypotheses will be tested. Finally, the variation in each test over time will be studied in relation to early and final diagnoses and course of therapy. At this point, far more sophisticated mathematical statistical methods, drawn from random walk and other stochastic theory, will need to be developed to describe dynamic changes in test results during hospitalization. It is anticipated that a series of publications will result from this collaborative research project.

Honors and Awards: None

Publications: None

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ANNUAL REPORT
(July 1, 1965 - June 30, 1966)

DIVISION OF RESEARCH FACILITIES AND RESOURCES

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National Institutes of Health
Bethesda, Maryland 20014

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ANNUAL REPORT
(July 1, 1965 - June 30, 1966)

DIVISION OF RESEARCH FACILITIES AND RESOURCES

NATIONAL INSTITUTES OF HEALTH

Dr. Thomas J. Kennedy, Jr., Chief

INTRODUCTION

The start of Fiscal Year 1966 marked both the third anniversary of the Division, established July 1962, and a change in leadership as Dr. Thomas J. Kennedy, Jr. assumed the responsibilities of Chief. Of necessity, great emphasis during this period was placed on recruiting for the key position vacancies that attended staff reassignments to NIGMS.

Intensive Division efforts resulted in successful recruitment of a new administrative team in the Office of the Chief. This included the Assistant Chief, Dr. Thomas G. Bowery; Executive Officer, John De Vierno; Grants Management Officer, Kenneth Anderson; Administrative Officer, Kent A. Smith; and Information Officer, James Augustine.

During the fiscal year the Division continued its efforts to unify its programs and to improve administration of its program responsibilities.

OFFICE OF THE CHIEF

Office of Program Analysis

During Fiscal Year 1966 the Program Analysis Office made substantial progress in the area of increased automation, streamlining the data capture mechanism, and staff training. The internal structure of the Branch was reorganized, with emphasis on individual staff responsibility for specific programs of the Division. Systematic quantity control procedures were developed and implemented to ascertain and improve the reliability of the data produced. Mechanical edit programs were instituted and a manual of procedures started. As a result of these measures, data production was increased and the quantity of output improved despite reduced staff.

As a part of the automation program, there was substantial emphasis on replacing manual computation and keypunching by electronic computer utilization. A program was written to calculate 1966 awards under the general research support (GRS) program, previously a manual operation. Other programs were written to produce statistical data for an analytical report prepared by the Office of Program Analysis, regarding the implication of alternative changes in the GRS formula. For the first time, statistical tables providing profiles of expenditures under the GRS program were successfully produced by computer.

Other activities included the design of a survey to collect needed information regarding utilization of health research facility (HRF) projects constructed with Title VII, Part A, funds. Technical consultation was provided from time to time to others in the Division and in NIH regarding sampling problems, coding systems, statistical analysis, and computer programming.

Office of Architecture and Engineering

The Office of Architecture and Engineering continued to guide grantees in the design and construction of their health research facilities with the intent of assuring maximum dollar value and the best obtainable facility to satisfy the research requirements specified in the grant application.

Substantial staff time is devoted to consultations with grantees prior to application and again during the intermediate design period. Questions on technical design and cost evaluation are prime subjects. Additional Laboratory Design Notes, the series of pamphlets devoted to improved laboratory design, were prepared this year. Approximately 13,500 copies on 25 subjects were distributed during Fiscal Year 1966.

Consultation services, plan review, and cost analysis are also provided for the larger alteration and/or renovation projects financed by research grants and research contracts. A brief guide for alteration and/or renovation projects was drafted. The preparation of a more complete guide is in process and this revision is based on the result of two years experience with such projects.

Successive reviews at the various planning phases of projects complicate an explanation of the staff workload. Approximately 30 percent of the total projects submitted for Council approval receive only initial review due to an unfavorable recommendation by the Advisory Committee or Advisory Council. The remaining projects undergo evaluation two or three additional times.

One hundred and thirty-nine sets of plans were submitted and reviewed by this staff in FY 1966, the health-related research space involved 3,600,000 net square feet at a cost of $220 million. The total cost of the facilities involved was $543 million.

In FY 1966, 51 sets of intermediate plans were reviewed which describe 1,500,000 square feet of health research space. The estimated cost of the space was $88 million.

Ninety sets of final bidding documents for construction contracts were reviewed and approved in FY 1966. These provided 2,400,000 net square feet of health research space at an estimated cost of $130 million.

Additional streamlining and simplification of the review process is being instituted as the competence and skills of the staff improve with experience.

The review and evaluation of construction costs conducted by this office is a sensitive administrative process. However, it was possible during FY 1966, to conserve $600,000 as a result of these control measures, and reallocate such

money to fund projects which otherwise would have gone unpaid.

Information Office

The Information Office continued to provide the branches, media, and various publics with a variety of needed information materials and tools. Information on Division programs was provided to LIFE, MEDICAL WORLD NEWS, LOOK, ARCHITECTURAL RECORD, NEW YORK TIMES, and EDP WEEKLY and also to several local and network radio and television stations.

During this fiscal year a brochure on the National Regional Primate Center program was initiated. An exhibit was developed for the Animal Resources Branch and other visual materials were prepared on Division activities in the development of a visual resource program to serve a variety of information activity needs.

A half-hour color TV program involving a research study at the Tulane Regional Primate Research Center was produced during this fiscal year. The program will be aired over the ETV network in the fall as one of eight programs in a science series devoted to diverse research projects.

ANIMAL RESOURCES BRANCH

The Animal Resources Branch administers the national primate research center program comprised of six regional and one national center. The Branch also administers grants, funded from other sources, for central laboratory animal facilities and other institutional animal resources, and for related research and training activities.

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National Primate Research Center Program

The national primate research center program provides seven unique facilities where studies are conducted using primates to advance biomedical knowledge in many disciplines.

By the end of FY 1966, construction of all seven centers will be completed. The Yerkes Regional Primate Research Center was dedicated in October 1965, and the National Center for Primate Biology and the New England Regional Primate Center will be formally dedicated in the fall of 1966. This program, then, enters the next fiscal year with all seven centers occupied and with active research programs being developed.

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Significant Problems

Program development of the primate research centers continues to be limited by funds made available to the program.

The need to provide construction funds for additional facilities at the primate research centers is still an unresolved problem. The centers were constructed by non-matching grants, the authority for which has since been

rescinded. The feasibility of construction by contracts is being explored. At the present time the only means of additional construction for auxiliary facilities and needed additional animal colony space, enlarged quarantine and isolation quarters, and other supporting activities is on a 50-50 matching basis, an arrangement not acceptable to the host institutions because of the regional and national character of the program.

Trends

Coordination and cooperation between primate research centers, and between the centers and other related activities, both Federal and private, continues to improve. Workshops are being conducted in colony management, storage and retrieval of biological information, and other activities of common interest, and information is disseminated to interested scientists throughout the country. Through the Institute of Laboratory Animal Resources, National Academy of Sciences, an interagency coordinating committee has been established to further aid in the rapid dissemination of information and coordinate research activities related to the use of the primate and other animals in medical research.

In FY 1965, there were two primate research center directors' meetings to discuss administrative problems and to coordinate program developments and activities.

Changes and Improvements (Administrative)

Close coordination has been maintained with the Financial Management Branch, NIH, in the development of policies pertaining to the funding of the primate research center. It is anticipated that such policies will be finalized shortly and the Policy and Administrative Procedures Guide for the Centers will be ready for publication during the first quarter of FY 1967.

Annual Budget negotiation meetings with Center Directors, formerly conducted at the centers, are now being held in the Office of the Chief, ARB, DRFR. This has been valuable not only in conserving the time of the limited ARB staff, but in allowing the participation of other NIH staff concerned including grants management personnel, Financial Management Branch representatives, and administrative representation of DRFR.

Future Objectives

The two primary objectives of this program are to:

1. Obtain sufficient funding to allow for an orderly and progressive development of the center scientific programs.

2. Provide adequate funding support for the construction of necessary supporting facilities.

In addition, the further coordination and cooperation with other institutions will be nurtured, and problems related to sources of primates and their care and management in a colony environment will be given increased emphasis to assure an adequate number of healthy animals available for biological studies.

Animal Resources Program (other than Primate Research Centers)

The general purpose of the animal resources program is to stimulate and assist in implementing improved laboratory animal facilities and resources for health-related research, nationally.

The Branch professional staff has continued to advise grantee and federal institutions about planning laboratory animal facilities and developing animal colony management procedures.

The Branch administers grants funded by other sources for new or renovated animal facilities in medical schools, for studies of development and breeding of species or strains of animals that show promise as valuable research tools, for the maintenance of strains of animals uniquely suited to certain kinds of research such as tissue transplantation, and for research on some major diseases of laboratory animals.

The Institute of Laboratory Animal Resources, National Academy of Sciences, through funds provided in part by the National Institutes of Health, is developing standards on the commonly used animals in biomedical research and disseminating information on laboratory animal medicine to the scientific community.

The Branch was a focal point for information on pending legislation dealing with laboratory animals.

Significant Problems

Of primary concern is the need for construction and renovation funds for institutional animal resources, necessary funds to support studies on animal care, colony management, diagnosis and control of diseases of laboratory animals, and development of special or new strains showing promise as biological models.

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It is estimated that 50 percent of institutional animal facilities need assistance to improve their animal facilities, and the presently authorized 50-50 matching funds requirement cannot be met by those institutions which most need help. There is also an immediate need for funds for the training of professional staff for animal facilities and to conduct studies related to care and management of laboratory animals.

During the year it has become apparent that legislation will be passed which relates to the control of laboratory animals. The form of legislation which is passed will have a direct bearing on the future funding requirements and development of this program.

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Trends

There is increasing awareness by institutions of the importance of animal facilities, hence a growing demand for funding support and technical/professional assistance in developing plans and related programs.

Concurrently, the momentum for federal legislation further emphasizes a growing recognition and concern for improved laboratory animal medicine programs which are essential in support of the Nation's medical research effort.

Future Objectives

The objective of this portion of the ARB program is to further develop a program which effectively supports this Nation's needs for laboratory animal facilities and resources, to include related research, training and technical assistance.

Table 1

PRIMATE CENTER PROGRAM

Type of Application	Number Received	Amount Requested	Number Approved	Amount Approved FY 1966	Amount Approved Future FY	Number Funded FY 1966	Amount Funded FY 1966
New	-	$ --	-	$ --	$ --	-	$ --
Renewal	2	2,431,460	2	--	2,431,460	-	--
Supplemental. . . .	4	1,131,205	4	842,137	--	2	45,303
Non-competing . . .	7	8,230,577	7	8,230,577	--	7	6,954,697
TOTALS.	13	$11,793,242*	11	$9,072,714*	$2,431,460*	9	$7,000,000*

* Includes Indirect Costs

Table 2

ANIMAL RESOURCES PROGRAM

Type of Application	Number Received	Amount Requested	Number Approved	Amount Approved FY 1966	Number Funded FY 1966	Amount Funded FY 1966
New	24	$2,865,516	13	$1,127,447	5	$ 533,851
Renewal	2	44,000	-	--	-	--
Supplemental. . . .	-	--	-	--	-	--
Non-competing . . .	15	591,191	15	591,191	15	456,804
TOTALS.	41	$3,500,707*	28	$1,718,638*	20	$ 990,655

* Includes Indirect Costs

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ARB NEGOTIATED CONTRACTS

Table 3

Contractor	Number Negotiated	Amount Requested	Amount Approved
Nat'l Academy of Sci. 2101 Constitution Ave. Washington, D. C.	1	$25,000	$25,000
TOTALS	1	$25,000	$25,000

GENERAL CLINICAL RESEARCH CENTERS BRANCH

The general clinical research center provides an ideal setting for the complex and exacting requirements of first class clinical research. In the controlled multidisciplinary environment of the center the clinical scientist is able to conduct the precise studies essential to the acquisition of new medical knowledge and to profit from the stimulus of sharing ideas with the scientists in other biomedical disciplines.

The centers provide a resource which raises the level of excellence and increases the quantity of research conducted. Findings from studies in these centers have made possible improved patient care. In addition, the quality of clinical teaching has been enhanced because of the center resource, and the center has served as a training ground for new scientific research workers.

Accomplishments

In the first six years of the general clinical research centers program 84 centers have been approved and five others have been authorized.* Of this number, 80 centers have opened their doors, six during Fiscal Year 1966.

Outstanding Research Conducted in 1965

With an average of 30 ongoing research projects at each of the centers in the past fiscal year, only a few can be selected to illustrate the broad range of studies.

Research workers at the University of Colorado center are attempting to minimize the disability in patients with pulmonary emphysema. Through use of a readily-portable liquid oxygen system the patients' exercise-tolerance and arterial blood oxygen have improved so that they require fewer phlebotomies. The success of this technique in returning these patients to an ambulant life has been most encouraging.

* Approved but not awarded.

Another ongoing project at this center has provided encouraging results with kidney transplantation. Of 46 cases where kidneys were supplied by related donors, 31 recipients (67 percent) survived for at least one year, and 15 (33 percent) have survived from 24 to 40 months. A new lymphocyte typing technique for donor and recipient is being utilized in an attempt to improve further these results.

At the University of Washington center, in Seattle, a method of extracorporeal irradiation has been devised by an artificial kidney research team and a group of engineers. Patients with leukemia have had dramatic lowering of their white blood cell counts by this method of treatment. In addition, since irradiation by this method almost selectively destroys lymphocytes, it is being employed as a tool to lengthen the survival of transplanted kidneys.

Another important study in progress at the University of Washington center is concerned with infants born with central nervous system defects. To manage the complex rehabilitation problem this presents, orthopedic surgeons, radiologists, psychologists, social workers, and pediatricians have teamed together. It has been found that medical and educational costs are reduced by this method of management.

Research workers at the University of Miami, Florida, have discovered a new type of mental retardation in which there is a two hundred-fold overproduction of uric acid with deposits of uric acid in brain tissue. This important finding has opened the entire area of how purine and nucleic acids relate to function of the nervous system.

In a mental retardation study at the University of Florida, Gainesville, a group of investigators have developed a simple bone marrow test which will permit early diagnosis of Hurler's syndrome. Large doses of adrenal cortical hormones have been found to reduce markedly the excretion of acid mucopolysaccharides in this disorder which causes mental retardation and physical deformity.

A valuable mode of treatment for management of patients with bronchial asthma has been devised by scientists at the Peter Bent Brigham Hospital, Boston. By giving single doses of prednisone on alternate days, the side effects of long-term steroid therapy have been abolished. Results of these studies will minimize serious complications in a wide variety of diseases in which cortical steroids are indispensable.

Research workers in a project in a general clinical research center at the University of Pennsylvania have demonstrated that certain individuals have increased hair loss because of a specific amino acid enzyme defect associated with excessive excretion of arginine and succinic acid in the urine. This offers a new approach to the rational treatment of unexplained thinning of the hair and brittle fingernails.

Investigators at the Philadelphia General Hospital have found that vitamin E deficiency may be the cause of anemia. It is hoped that correction of this anemia will prevent serious problems associated with prematurity, such as mental deficiency.

Research workers at the University of Michigan general clinical research center have developed a method to measure externally the battery levels of the implanted heart pacemakers, the resistance between the electrodes to the heart, and the threshold energy required to stimulate heart action at a given time. This is an important contribution since previously function of these electrodes could be evaluated after implantation only by making a new incision in the chest and taking direct electrical measurements on the pacemaker wires.

Concurrent studies by scientists at Yale and the University of Michigan have focused on preventing the death of babies affected with erythroblastosis fetalis. At Yale, a new optical instrument has been developed which permits direct visualization of the blood vessels for in utero transfusion. At Michigan a new diagnostic test has been developed to indicate which erythroblastosis babies are in difficulty; transfusing these infants in utero has reduced their mortality rate by 50 percent.

Because the illnesses seen in the clinical centers are studied in great depth, they have been a unique source for training at all levels of medical career development. In many centers, conferences have been established which are attended by paramedical personnel including social workers, dieticians, occupational therapists, as well as medical specialists, where patients' illnesses and problems are discussed.

Significant Problems

The problem of overpayment of hospitalization by the 85-15 formula appears to have been resolved. The basis was use of the 85-15 formula for the first 12 months of operation and a cost formula for the subsequent periods.

Future Objectives

It is expected that many of the existing medical schools which do not now have such a clinical resource, as well as the newly established medical schools, will want to have general clinical research centers. A number of the Divisions' advisors have expressed the view that a general clinical research center should eventually be established in every medical school.

A program need exists also for an analagous resource for ambulatory subjects similar in concept to the inpatient general clinical research center. At present, research in ambulatory subjects is largely observational. This means a large number of important studies such as therapeutic trials on ambulatory patients with adequate scientific supervision are neglected. Outpatient facilities would not only permit studies not now feasible, but would also improve many investigations currently performed in inpatient centers. Coupling ambulatory facilities with existent inpatient centers would reduce the cost of the individual research project as well as relieve the pressure for increased expansion of many centers.

An expansion of the present general clinical research centers program to include support of discrete ambulatory clinical research centers as institutional resources would substantially contribute to an increase in clinical research productivity.

BUDGET DATA

		Amount
Fiscal Year 1966 Appropriation		$28.5
Awards for FY 1966		
	No. of Centers	
Continuations, requiring funds	79	$26.6
Continuations, not requiring funds until FY 1967*	4	0
Supplements	(7)	.5
New Awards	1	.7
Total	84	$27.8
Unpaid March 1966 NARRC Approvals		
5 New		2.6**
3 Supplements		.2**
Indirect Cost Estimate		.2
DEFICIT		(2.3)

* Awarded in FY 1965
** Exclusive of Indirect Cost

CUMULATIVE STATUS AND TYPES OF GENERAL CLINICAL RESEARCH CENTERS

FY 1960 - 1966

Type of Center	Cumulative Status of Centers						
	FY '60	FY '61	FY '62	FY '63	FY '64	FY '65	FY '66
Adult [a]	8	31	43	48	55	58	58
Children's		1	8	13	16	18	19
Adult-Children's [b]			3	3	3	3	3
Premature			1	1	1	1	1
Acute			1	1	1	1	1
Chronic				1	1	1	1
Maternal-Child					1	1	1
Totals	8	32	56	67	78	83	84 [c]

[a] Adult Centers may also admit children.
[b] Consists of separate adult and children sections under one program director.
[c] As of May 1, 1966. Five additional centers approved but not yet funded.

GENERAL RESEARCH SUPPORT BRANCH

The stimulus for the General Research Support Program had its origin in the post-war growth of the National Institutes of Health's extramural research support program.

In recognition that support of research exclusively through the project system had deprived educational institutions of a large measure of autonomy and freedom in determining the character and direction of their research activities, and that exclusive reliance upon the project system precluded educational institutions from assuming full responsibility in the conduct of their health-related research activities, the General Research Support Program was initiated.

Program Goals

In its objectives and policies, the General Research Support Program encourages institutions to meet emerging opportunities in research to explore new and unorthodox scientific ideas and to use research funds in ways that will contribute to long-range institutional development for the improvement of research. Included in the program goals are: 1) stabilization of key research personnel; 2) support of new or exploratory research projects not ready to compete successfully in national competition for NIH project funds; 3) support of central, institutional resources such as computer centers and animal facilities.

The general nature of the program enables institutions to exercise greater scientific responsibility over the emphasis, direction, content, and quality of an entire research effort, or specific parts of it.

Accomplishments

In fiscal year 1965, the Congress appropriated funds ($5 million) to extend the program to academic institutions other than health professional schools, provided the former were able to demonstrate a serious engagement in biomedical research and research training.

It is a fact that a significant proportion (approximately one-fifth) of extramural research project support funds distributed by the NIH go to academic institutions other than the health professional schools. Therefore, in recognition of the important role which these institutions play in the development of medical science and in the training of biomedical investigator it was considered appropriate that general research support be extended to this segment of the nation's basic educational resources.

This extension, under the title of Biomedical Sciences Support Grant Program was launched in April 1966, and approximately 100 applications have been received and reviewed to date. The program is designed to provide the same type of flexible long-term support to the universities which has been provided to the health professional schools.

Also inaugurated in fiscal year 1966, was the Health Sciences Advancement Award Program. Initially, this program will assist universities and health professional schools, and at a later date possibly other types of health-research organizations, in the support of well-conceived, broadly based research and research training programs in the health-related sciences.

The objectives of this program are to accelerate the advancement of existing capabilities in health research and research education within institutions which already have demonstrated some competency in the biomedical area; support the advancement of new health research and training endeavors in emerging and less well established academic institutions which possess an appropriate base for future development; and promote the better training and increase the number of biomedical investigators. Awards are competitive in nature, non-renewable, and will range from approximately $1 to $3 million over a single 5-year period. Two awards were made under the approved $1 million budget in fiscal year 1966.

In fiscal year 1965, 273 General Research Support grants were awarded to 198 institutions, the awards ranging from $10,271 to $585,064 with the median grant being $105,630. The annual progress reports required for this program cover the calendar year and thus will not be fully analyzed until after June 30. It is likely, however, that the major trends observed in fiscal year 1963 and fiscal year 1964 will again obtain.

To date, the results of the General Research Support Grants program in support of institutions as contrasted with the support of individual researchers have been endorsed with enthusiasm by several advisory groups which have evaluated the General Research Support Program. The Branch, within the constraints set by available funds, looks to providing awards to additional institutions which meet eligibility requirements in the years ahead and to increases in the size of awards made to eligible institutions.

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Significant Problems

Since the beginning of the regular General Research Support Program in 1962, there have been five annual cycles of application, review and approval, and award. In addition, progress reports on three years of experience have been analyzed. A part of this experience was reviewed at the request of the National Institutes of Health by a committee organized by and responsible to the National Academy of Sciences/National Research Council. Out of these analyses, several areas have been highlighted as worthy of careful exploration with the possibility of policy revision in mind. The National Institutes of Health is now in the process of examining these issues, reevaluating the extent to which the present policy serves the intent for which it was adopted, and weighing the comparative significance of the consequences of maintaining the status quo, or changing to a new policy. Several specific instances may be mentioned.

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A. The architects of the program placed a premium on those non-Federal funds restricted at the time of donation for the support of health research activities and utilized by institutions for this purpose, in order to demonstrate clearly the Federal

government's intent not to supplant private support with Federal grants and contracts. Any stimulation in the rate of growth of non-Federal research expenditures which occurred as the result of this policy was considered to be of secondary importance. Analysis of existing data has indeed highlighted the difficulty of assessing the extent to which this policy has induced significant differential incrementation in non-Federal spending. However, serious administrative problems have been and continue to be encountered in verifying claims to qualify such non-Federal expenditures as a basis for computing the magnitude of an award. The unusual extent of administrative difficulty recommends eventual elimination of non-Federal research expenditures from the formula for calculating the size of the GRS award. However, it is clear that the transition from present procedures to any proposed alternative must be engineered so that major and sudden perturbations in the budgets of grantee institutions are avoided.

B. The wisdom of continuing indefinitely the present privilege of automatic eligibility for GRS support for certain classes of health professional schools has been questioned by both the NAS-NRC Committee and by the GRS Scientific Advisory and Review Committee. The basis for the reservations held by these groups is a desire to base the regular GRS award on merit. Continued extension of eligibility to institutions unable to meet minimal qualifying requirements imposed on non-privileged institutions may be considered to be equivalent to the award of developmental funds. While enthusiastically in favor of the latter type of award, the advisory bodies appear to prefer that programs for institutional development be kept separate and distinct from those which reward merit and achievement. Any policy reorganization on this issue of automatic eligibility must be evaluated in relationship to the consequences of the loss of support to affected institutions and the present or possible availability of other mechanisms, e.g., the establishment of additional, time-limited developmental award programs designed to take over certain support activities now funded through the GRS mechanism.

These are a few of the many problems which have emerged in the course of actually operating the General Research Support Program.

Future Objectives

The National Institutes of Health has sought, through a variety of mechanisms, to stabilize the pattern of salary support for research workers in academic and other biomedical research institutions. The objective is to create a more effective partnership between the National Institutes of Health, with its statutory missions, and the centers of scholarship, education, and research. The most recent venture into this type of effort was the Research Career Program. Due to operational difficulties as experience accrued, the further development of the senior category of this program was discontinued in 1964. The objective of this program--namely, the stabilization of salary support for established investigators--remains a valid one, and can properly

be viewed as an element in the pattern of general support for research extended to an institution. In the redesign of a program directed toward this end a more workable arrangement might be possible under the General Research Support Program.

The Woolridge Committee accepted the recommendation of its Panel on Administration that the National Institutes of Health negotiate the salary items that presently are funded through a whole series of individual research and research training project awards directly with the institutions, as a single contractual arrangement.

Conclusion

The concept of providing flexible, general research support for institutions engaged in biomedical research has evolved not only from long discourse between the Federal Government, investigators, and organizational entities, but from the crucible of experience. In practice, the first phase in the implementation of the concept has been done on a deliberate basis, building carefully at each step on the knowledge already acquired. Extension this year of general research support to the non-health professional school sectors of universities represents, for this facet of the use of the overall General Research Support authority, a logical and necessary culmination to the specific program initiated in 1962. The total scope and flexibility of the General Research Support authority, however, provides an unprecedented opportunity for developing additional, meaningful forms of support for the Nation's biomedical research organizations, by which means it should be possible to improve and extend their current and future capabilities in the health sciences, and to increase the total numbers of strong centers for research and research training.

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HEALTH RESEARCH FACILITIES BRANCH

The health research facilities program is now in its tenth year. The program is designed to advance biomedical research through the provision of matching funds for the construction of urgently needed, functionally designed, and modernly conceived research buildings. Such funds may be used for the construction of new, and/or substantial renovation of old, research buildings, together with the equipping of such structures.

Since Fiscal Year 1964, there actually have been two distinct programs. One, operated under Title VII A of the Public Health Service Act, provides for grants of up to 50 percent of the cost of biomedical research facilities and equipment, committed by the institution to such use for a minimum of ten years. The second, operated under Title VII D of the Act provides for grants of up to 75 percent of the cost of facilities uniquely designed for research in mental retardation and committed to such purpose for a minimum of 20 years.

As the research facilities program entered its tenth year it became evident that its character has changed gradually with the result that the present program has placed strong emphasis on the provision of thosefacilities which will provide broad institutional support for all aspects of biomedical research. Concomitant with this has come a considerable increase in the size of the average grant--thus, grants made during this most recent fiscal year average $667,605. Since such awards represent at most 50 percent of the construction costs, and more frequently considerably less, due to the need for multipurpose buildings, the cost of the total facility is in the range of $1.5 to $5 million. This has required exceedingly close cooperation with the recipient institution, in order to meet both the programs' goals and the unique requirements of the grantee institution. Further, during the past several years the multipurpose facilities which have been supported, frequently have incorporated large areas for the teaching of health professional personnel. This, in turn, has required close cooperation with the Division of Hospital and Medical Facilities--the Public Health Service unit which has responsibility for operation of the teaching facilities program under Title VII B of the Act. A consequence has been a careful assessment both of the Nation's needs for increased research facilities and the institutions' desire to provide these, together with its potential for the effective use of such components in the conduct of biomedical research. Only when agreement has been reached that such potential does exist is an award made.

During the past year, a project site visit has been made to each institution which has requested support. A total of 130 applications were presented to and reviewed by the Health Research Facilities Scientific Review Committee and the National Advisory Council on Health Research Facilities. In addition to these formal gatherings, there have been innumerable consultations with representatives of interested institutions. The end result of these activities has been a considerable improvement in the applications which have been submitted, an increased awareness of the program goals on the part of the applicants, and a more sharply defined focus in the awards which have been made. The true value of all of these efforts will not be apparent for several years, however, due to the inherent lag in construction programs occasioned by the 12 to 18 month period required for actual completion of most larger facilities following

award of construction contracts.

Accomplishments

The accomplishments of a construction program cannot immediately be seen. The results of awards given this fiscal year will not be visible for several years beyond the date of this report. It is possible only to specify numerically the starts which have been made. The impetus which has been given and the directions that have been taken can be demonstrated only with the passage of time.

In FY 1966 there were 72 awards for $51,012,393 made under Title VII A (as of May 26, 1966).

Among the most meaningful of these awards were seven grants to provide research facilities in new medical schools. By providing assistance for the construction of research facilities in these newly developing medical schools, the National Institutes of Health has enabled their administrations to attract the highest quality of staff.

These seven awards were for the following institutions:

The University of Arizona Medical School at Tucson, $1,161,500; the University of Connecticut Medical School at Farmington, $5,900,000 (this includes research areas for the new Dental School); the University of Hawaii Medical School, $1,152,000 (this includes facilities for the new School of Public Health); the University of California at San Diego, $1,560,000; the Pennsylvania State University Medical School at Hershey, $1,711,800; the Brown University Medical School at Providence, $1,110,500; and the University of Texas South Texas Medical School at San Antonio, $1,437,700.

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These grants, which totalled $14,033,500, represented about 28 percent of the current year's obligations.

Other awards of prime importance included a group which represented provision of major new facilities for research activities in connection with the relocation and/or expansion of existing medical schools.

There were six awards in this grouping as follows:

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University of Louisville, Kentucky, $2,703,000 for research facilities in the basic sciences; University of Michigan at Ann Arbor, $2,545,000 for research facilities in the medical sciences; University of Puerto Rico, $1,767,500 for new research facilities in the relocated school (also includes facilities for dental and public health research); St. Louis University Medical School, $1,369,000 for new basic science research facilities; Washington University, St. Louis, $2,370,700 for new basic science facilities; and the Western Reserve University, $1,459,000 for new research facilities in anatomy.

These awards, which totalled $12,214,200 and represented nearly 25 percent of the fiscal year obligations, will permit each of these institutions to upgrade the caliber of their teaching and research staff, and improve their

research programs through provision of modern, functional research laboratories.

In a similar manner assistance was given to two dental installations for major expansion of research facilities. These awards were $1,102,000 to the Forsythe Dental Center in Boston, Massachusetts, and $1,443,900 to the University of Pennsylvania School of Dental Medicine. In each instance the proposed research expansion was coordinated with a major effort of the National Institute of Dental Research to expand the dental research potential of our Nation. A similar award was for $2,470,000 to the School of Public Health of Harvard University, for a major expansion in their facilities, especially for research in tropical public health, in microbiology, and in population studies.

It can be seen, therefore, that nearly 65 percent of the funds available during this fiscal year have gone into research facilities which represent major institutional resources. This represents a very significant trend which we believe will continue for the next several years.

As in past years a number of new and remodeled facilities were completed and placed into use. There were 59 such facilities during this fiscal year with a total cost of $150,132,105 of which $30,342,211 represented Title VII A funds.

Among the institutions that opened their doors during this period were:

The new University of Utah Medical Center formally dedicated on October 16, 1965, although it had been placed in use some months earlier.

The Cell and Virus Research Building of the Roswell Park Memorial Research Institute at Buffalo, New York, dedicated on October 21, 1965.

The Botany and Microbiology Building at the University of Oklahoma, Norman, dedicated on October 22, 1965.

The groups of five new Science Buildings at Brandeis University, Waltham, Massachusetts, dedicated on November 7, 1965.

The Clinical Research Building of the University of Tennessee, Memphis, dedicated on November 13, 1965.

The Black Research Building of Columbia University, a 20-story research tower, dedicated on January 3, 1965.

The Laboratory of Clinical Investigation at Yale University, dedicated on April 16, 1966.

The Henry L. Moses Research Institute of the Montefiore Hospital, New York, a strikingly beautiful research tower, dedicated on May 5, 1966.

Each of these splendid new structures represented the fruit of a joint effort between public or private institutions and the Government, to provide research facilities for the rapidly expanding biomedical enterprise.

The mental retardation research program also continued to progress during this year and four awards, totalling $6,234,200 were made as follows:

The University of California at Los Angeles, $1,660,000; the Children's Hospital Medical Center at Boston, Massachusetts, $2,470,000; the University of Kansas for facilities at Kansas City and Lawrence, $1,758,000; and the University of Kansas in cooperation with the State Department of Social Welfare, $346,200 for a facility at the Parsons State Hospital. As was the case in prior years, each of these awards was preceded by a long period of intensive planning and collaboration between the National Institute of Child Health, the Division of Research Facilities and Resources, and the grantee institution in order that the proposed facility might represent the best possible solution to the need for a comprehensive mental retardation research installation in the geographical area selected.

There is considerable emphasis on the development of large institutional resources, particularly in connection with establishing new schools of the health professions and expanding existing schools. Such commitments have taken almost 65 percent of the current funds. At the same time it is necessary to maintain a program balance through consideration of these institutional needs, as related to the expansion of health manpower, and the institutional requirements that are necessary to permit research programs of significance to the Public Health Service and the Nation's health. Within the monetary limits set by the Congress during this past year, it has been possible through this fiscal year to maintain such a balance.

Significant Problems

These continue to be the same as those mentioned in prior years. First of all is the inability to promptly fund all requests recommended for approval. Second, is the requirement that the federal share not exceed 50 percent. This restriction has greatly hampered the development of animal resources, expansion of the primate research center program, developments in environmental health, and research programs in pharmacology and toxicology. At this writing there is pending in the Congress a measure which would permit a relaxation of this restriction; in the case of animal facilities the federal share could go as high as 65 percent. It is impossible to predict whether this bill will be enacted at this session.

Future Objectives

Within the past several months there has been a greatly increased effort on the part of many individuals to strengthen existing laws relating to the care and use of research animals. A prime consideration in improving animal care is the provision of adequate housing. As a consequence, it is evident that a considerable portion of the health research facilities funds to be spent within the next several years will be used for such purposes. Such usage will, of course, merely continue a trend which has been evident since the start of this program. Of the first seven grants which were awarded in November 1956, three were for animal facilities which were urgently needed. Since that time the proportion has increased so that, at times, as much as 20 percent of the funds awarded have been used for animal research facilities.

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Another continuing objective will be the development of institutional research facilities. This trend is now well advanced and it gives every evidence of continuing for the next several years. Although the establishment of new medical schools no doubt has passed the peak for the present, the needs for research facilities in expanding schools will continue for the next decade.

A third objective which has continued unabated during this past year has been the improvement in grants management procedures to insure the highest possible return on the federal investment. Included in this is the improvement of liaison efforts with other government agencies which frequently are involved with the Division of Research Facilities and Resources in the funding of multipurpose buildings.

Budget Data

Although presented previously in narrative form, a brief recapping of the pertinent budget data follows:

Funds available for grants:

$52,536,399 for Title VII A (represents appropriation of $50 million plus recovered funds from prior years).

$6,260,000 for Title VII D (represents appropriation of $6 million plus unobligated funds from prior years).

Applications received and amount requested:

50 for $31,966,233 (however, about 50-60 additional applications are expected by June 30, 1966).

Applications approved:

88 for $60,420,000 (about 50 of these were received by June 30, 1965, in FY 1965).

SPECIAL RESEARCH RESOURCES BRANCH

There has been increasing awareness of the significance of advances in the application of physical sciences and related engineering and technological capability to medical research and health services. During the past year the special research resources program has continued its role of accelerating the vital interplay between the life sciences and the technology and concepts of the physical sciences: 1) by establishing and expanding specialized institutional resources which meet a well-defined, demonstrated need within an institution or region; 2) by extending special research resource support into new areas of the biomedical sciences as the needs become apparent.

The broad areas of special research resource support have been computer and data processing centers, biomedical engineering, biomedical materials,

biochemical instrumentation resources, hospital data communication and control, and research in science communication.

A computer research resource provides the technical and professional staff and computer hardware to support computation and data processing for multidisciplinary and multicategorical health-related projects within an institution or geographical area.

Biomedical engineering brings to the health sciences the competence of the field of engineering to provide a broader-based scientific attack on medical problems.

Biomedical materials facilities provide specialized and hard-to-obtain biomaterials such as unusual enzymes, biopolymers, specialized plastics, microorganism and cell cultures, and marine organisms.

Biochemical instrumentation resources provide and support the development of increasingly sophisticated instruments required by today's biomedical scientists.

Research in hospital data communication and control is directed toward the application of automation and various analytic and diagnostic instruments to clinical settings. Research in science communication is directed toward furthering the dissemination of scientific information.

During this fiscal year, 51 applications were reviewed, of which 30 were recommended for approval at an amount totaling $6,250,294. This included six competing continuations in the amount of $2,085,341 which will be eligible for funds beginning in FY 1967 and FY 1968. The program currently administers grants for 64 special research resources, including 43 computer centers.

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Accomplishments

Dramatic and significant advances have been realized from the application of digital computers to the biomedical sciences. A digital computer has increased the accuracy and control in the radiation treatment of cancer and reduced unplanned overexposure and its attendant damaging effects. It is also permitting an attack on the problem of providing optimum conditions for radiation treatment for each patient. The speed with which biomedical computer centers collect, process, and evaluate data has made possible new conceptual approaches to difficult research problems years before such approaches might have been developed without the application of computers.

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The following studies are examples of the scope of research which the special research resource program supports:

At Northwestern University investigations are being made into characteristics of new materials from which improved artificial organs such as kidneys, lungs, and other patient-support systems might be constructed, as well as practical studies to evaluate a compact heart pump requiring only a small volume of blood to prime the system.

At the University of Pittsburgh a recent advance in analytical methodology, of great potential value for biomedical research, has resulted from the ability to combine mass spectrometry and gas chromatography for the direct recording of mass spectra of all organic compounds in a complex mixture. A mass spectrometric facility has been established for use in a variety of research projects at the institution and in cooperative programs with scientists at other institutions.

Support is being given to an intensive and sophisticated program of research aimed at the development of a time-shared, remote-access computer system in a general hospital. The program, underway at Massachusetts General Hospital, is designed to increase the rapidity and accuracy of collecting, recording, transmitting, retrieving, and summarizing information on patient care activity in the hospital, and to facilitiate the storage of large amounts of complex medical information, permitting rapid and easy search and retrieval of the stored information for clinical research.

A common research computer facility has been established at the Texas Medical Center to serve as a centralized regional resource for medical, life, and computer scientists. This center satisfies the common computing requirements of the academic research and affiliated institutions in the Texas Medical Center, the Houston academic community, and various medical units of the University of Texas. The variety of research projects under investigation that utilize the resources at the facility range from simple descriptive or data reduction techniques to sophisticated simulation models of cellular growth and cardiovascular function.

Trends

The number of grants given for computer support will undoubtedly continue to increase for the next few years before leveling off. As major universities establish computer resources with their own or with NIH funds, the number of grants will taper off. As the present centers grow there will be a greater exchange of computer time and computer programs. Although the number of centers will become stabilized, the dollar support will increase with the number of investigators and with the sophistication of computer techniques.

Support of biomedical engineering is likely to expand substantially as a result of the recent recognition by health scientists and engineers themselves of the contribution engineering can make in solving health problems. This trend is evident in the increased number of investigators who, this past year, discussed with the Special Research Resources Branch staff their needs for support in bioengineering.

The number of specialized health-related resources, those providing cultures of living materials and biochemicals, is expected to remain fairly constant. Because industry can provide cultures of living materials and biochemicals, the special research resources program supports only the production of materials which are for any number of reasons impractical for industry to furnish. For instance, the quantity required by some investigators could be too small to be profitable.

At many research institutes, scientists working in the field of biochemistry need the capabilities of advanced analytical equipment to investigate the molecular structure of proteins and enzymes. Examples of the equipment involved are mass spectrometers and nuclear magnetic resonance spectrometers. As the competence and sophistication of the biochemical investigators grow, the special research resources program will expand to meet these increased requirements for analytical biochemical resources.

Support of hospital data communication and control is likely to expand because of the increasing requirements of basic research in the clinical setting; the application of automation and instrumentation to real life situations in hospitals; and the need of the clinical scientist for ready access to and evaluation of large amounts of complex medical information.

Support of research in science communication will probably be limited because sources of competence within the country are relatively few. The cost increase of the program will be largely for support of computer data processing facilities and the cost of acquiring and maintaining collections of document references on cards or tape for the use of investigators.

Administrative Changes and Improvements

The annual progress report for Special Research Resources Branch grantees has been revised and will be given a trial run with all SRR grants having an October 1, 1966 anniversary date.

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SPECIAL RESEARCH RESOURCES BRANCH
Total Awards and Funds Available

July 1, 1965 - June 30, 1966

	Awards	Available
Grant Funds		$12,150,000
New	$2,995,489	
Competing Continuations	262,800	
Non-competing Continuations	7,755,802	
Supplements	1,131,809	
Total		$12,145,900*
Estimated Balance		$ 4,100
Collaborative Studies		
New England Enzyme and Biopolymer Resources at Tufts University	116,525	
National Academy of Sciences	96,100	
Institute of Laboratory Animal Resources, National Research Council	25,000**	
University of Utah	5,000	
University of California, Los Angeles	23,000*	
Total		$ 265,625
Balance, Collaborative Studies		$ 49,375

*All data are actual awards except for one new grant currently under negotiation and estimated indirect cost adjustments on two awards.

**Currently under negotiation.

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HIGHLIGHTS

The Division of Research Grants participates in coordinating policies and procedures for Public Health Service grants and awards, and processes and provides scientific review for applications for research support. Established in 1946, it has completed 20 years in its role of assisting the scientific community in furthering health-related research.

This year, the DRG assumed additional responsibilities for review with the transfer of the Committee on Scientific Publications, the NCI Collaborative Research Panel, the Environmental Sciences Review Committee, the review functions of six panels of the U.S.-Japan Cooperative Medical Science program, and the creation of the Cerebrovascular Committee.

An increase in the complexity of grant applications and number of new programs, as well as the actual increase in the number of applications, led to the establishment of four new study sections for research grants and three new fellowship review committees, increasing these review groups to 60 and 15, respectively.

The Division completed an evaluation of a pilot study to increase the role of PHS-supported grantee institutions in managing research projects, and submitted recommendations to the Office of the Director, NIH, and the Office of the Surgeon General, PHS. The study, conducted in seven institutions from November 1964 until October 1965, employed experimental procedures for apportioning certain management functions to grantee institutions, and involved grant administrators and coordinators of the participating institutions in formulating project decisions in lieu of PHS staff. The conclusion was that increasing responsibility at the grantee level would "(a) relieve the PHS offices of a large burden of decision which is very difficult to carry out knowledgeably at a central point; (b) facilitate the changes in methodology and approach which are naturally inherent in research project management; (c) develop closer and more cordial working relationships between investigators and administrators in grantee institutions; (d) stimulate the continued development of research administrative capability in American universities and other scientific organizations; and (e) work toward full cooperation and mutual respect between Government and nonprofit institutions in the administration of Government-assisted research."

Dr. Eugene A. Confrey, Chief, served as executive secretary of the Surgeon General's task force in March 1966 to study and recommend organizational changes in grant and contract management responsibilities of the PHS. The task force recommended measures to restructure the total PHS organization for the broader responsibilities that the Service will assume in the years ahead.

Noteworthy policy changes during the year affected indirect costs under research grants, cost-sharing by grantee institutions, the conduct of clinical research and investigations involving humans, and the protection of the individual privacy of grantees.

OFFICE OF THE CHIEF

Dr. Eugene A. Confrey, Chief, was one of 12 recipients of the Department's Superior Service Award presented by Secretary John W. Gardner April 11,1966, for "important contributions to the research mission of the PHS through his highly effective leadership and administration of the Division of Research Grants."

At the request of the Director, NIH, Dr. Confrey headed the 1966 NIH-wide U.S. Savings Bond Campaign.

Staff on Special Detail—The Division provided senior staff assistance to the new Regional Medical Programs to develop policy and procedure for their grant and award programs.

A scientist administrator whose major fields of interest are anatomy and endocrinology completed a 2-year special detail with the World Health Organization in Geneva, Switzerland, to study human reproduction in relation to birth control.

The Division's Executive Officer assisted the National Foundation on the Arts and Humanities in developing its annual budget and establishing a grant program.

Internal Operating Guide—As a sequel to the PHS policy statements and guides for operating procedures for research and training grants, the Division plans on Internal Operating Guide for use by PHS extramural staff.

The guide is being developed,as a series of statements on staff procedures, to promote uniformity in administering the grant and award programs.

Office of the Associate Chief for Analysis and Statistics

During FY 1966, work progressed to improve the quality, coverage, and speed of accessibility of information about extramural programs of the NIH. Major attention was also given to new problems resulting from impending changes in computer equipment. The Office continued to provide liaison and operational contact for the DRG and the Office of the Director, NIH, on program analysis and reporting activities with other offices having related interests.

Progress and Plans for the Central Data System—In this first full year of computer-prepared award notices, a significant increase in the accuracy of keeping data current in the Central Data System has been achieved. The many timely and diverse reports prepared from the system, on a recurring basis and upon request, have been tailored to serve more exactly the needs of the institutes and divisions, and other users. Since July 1, 1965, about 400 requested reports have been supplied through the query program. The flexibility of the query routine—a generalized program designed to reduce programming bottlenecks—has been increased during FY 1966 to permit more types of reports in a 24-hour period.

Several major additons to the system have increased its coverage and versatility. One addition is a computer-based system for training grant programs. Information comparable to that maintained for the research grant programs is now being introduced and maintained for training grant programs. Reports and documents similar to those provided to institutes, divisions, and initial review groups for research grant programs will be provided for training grant programs.

Another addition is a computer-based system for information on trainees being supported under PHS research training grants and general research support grants. Data on trainees supported by FY 1965 funds are being captured and computer reporting is to begin in early FY 1967.

The Office collaborated with the Office of the Associate Chief for Research Analysis and Evaluation, DRG; the Office of the Director, NIH; and the institutes to develop the NIH Central Scientific Classification System. Classification of research grant applications and entering the codes in the NIH Central Data System began in February 1966. The data will be available through the query routine for rapid reporting.

Other classification structures being built into the Central Data System this year include institute program area codes, type of grantee institution codes (institution of higher education, hospital, etc.), and type of control codes (public or private).

Computer Conversion—The Office participated in a review of NIH computer needs which resulted in the NIH decision to procure a new, large-scale IBM computer. After the computer equipment was selected, the Office directed a 4-month joint systems study by personnel of the DRG, the Division of Computer Research and Technology, and IBM to redesign the DRG Data Processing System. The design incorporates advanced concepts of computer systems and makes optimum use of the new computer's random access storage and remote unit capabilities.

The new system has been implemented and conversion is scheduled for completion in February 1967.

Emphasis on Analysis and Projection —The Office focused renewed effort this year on analytical studies, and the use of statistical samplings and projections. Publication of Organizational Charts and Comments for Selected Grantee Institutions made available results of an extensive organizational study of about 100 grantee institutions. Other studies are reported by section under "Statistics and Analysis Branch."

Statistical sampling is being used to secure information which is not routinely captured as part of the Central Data System. Samples have been drawn from research projects supported with FY 1964 and FY 1965 funds. During FY 1967, samples of other NIH programs will be selected and analyzed.

Plans initiated in FY 1965 for budgetary projections based on data from the Central Data System were completed in FY 1966 and several comprehensive projections for research

grant programs were prepared. Projections are designed for use by NIH institutes and divisions and the Financial Management Branch in budget formulation and in analysis of budget trends and future financial requirements.

Office of the Associate Chief for Research Analysis and Evaluation

The Office of the Associate Chief for Research Analysis and Evaluation acts as a central resource within the DRG for analyzing the accomplishments, scientific trends, current support and future potential of research and training activities of diverse fields of health and health-related science.

Surveys and comprehensive studies are conducted, principally on disciplines and research areas that fall within multi-institute or division programs of support. Special reports describing the status of these fields are prepared for the Office of the Director, Extramural Programs; Chief, DRG; program directors in the institutes; and administrative management staff members.

The ORAE coordinates these requirements for scientific information with all available resources such as the Science Information Exchange, the National Library of Medicine, program analysis components in the institutes, the Research Documentation Section, the Federal Clearinghouse, and services in other agencies.

As an outgrowth of these activities, the ORAE has been developing new methodologies and general systems for documenting, communicating, and analyzing scientific information. Interagency coordination has been carried on for comparative program analysis and the exchange of information on research programs.

Sections Reorganized—To augment its capabilities, the Office was reorganized during FY 1966 into three sections: the Scientific Evaluation Section (SES), the Research Operations Support Section (ROSS), and the General Systems Research Section (GSRS). A concomitant increase in professional staffing will continue in the coming year.

A recently established evaluative unit for parasitology and microbiology in the SES has begun a comparative analysis of the research objectives and the actual research areas under investigation in projects supported by the NIH in the field of immunology. A detailed analytical model has been designed to illustrate their relationship.

The Biology Unit, SES, will soon complete and distribute an exhaustive analytical report on PHS support of research in nutrition. The scientific categorizations, administrative data and information in the report were discussed with members of the Nutrition Study Section at their January meeting. Further coordination at an April meeting provided additional comments and expertise to be included in the finished report.

The problem of determining the relationship of research grant funding to various population factors and to criteria for reviewing grant applications is being approached by the

General Systems Research Section. Much of the accumulated data has been correlated through computer analysis and interpreted with the aid of advance statistical methods.

The Research Accomplishments Unit, SES, continues to direct the Information Exchange Group program which consists of seven independent groups of 3,000 research scientists. One division and two institutes have consulted the Unit for information on setting up similar groups in certain areas of their programs. The ORAE was instrumental in organizing a Workshop on Bioengineering that met November 9, 1965, in Philadelphia, Pa. Proceedings of the meeting are being prepared for publication. The Associate Chief for Research Analysis and Evaluation was chairman of one of the workshop panels at this meeting, and the Chief, DRG, gave the opening address.

The ROSS has coordinated with institute program staff and numerous information sources on PHS support in a number of broad research fields. Data were gathered and preliminary analysis and interpretation given for institute reports covering Government-wide support of endocrinology and automation as applied to diagnosis, management, and therapy of disease. Similar coordination with the National Institute of General Medical Sciences resulted in a study of the support of research and training in departments of chemistry for FY 1964. An accounting of PHS grantee scientists active in research on transplantation during FY 1965 has recently been compiled and distributed to the Surgery Study Section as a resource of potential conferees for a national meeting on transplantation surgery.

New Technical Unit—A new activity in the ORAE is the Technical Publication and Documentation Unit which has catalogued hundreds of scientific, technical, and administrative reports on Federal support of research and training. Bibliographic references and, in some cases, entire documents such as the Westheimer Report on Chemistry have been provided on request.

During FY 1966, the ORAE Chief and staff members from two of the sections, acting jointly with the Statistics and Analysis Branch, developed a system of disciplines, medical specialties and body system categories for broadly classifying the PHS research projects to be introduced into the administrative record. This system, called the "NIH Central Scientific Classification Codes for Research Grants," was implemented in February. The ORAE is participating in followup studies of the problems encountered in indexing and the effectiveness of retrieval with this system.

With the consultation and guidance of the ORAE, one of the DRG grants associates has prepared an exhaustive analysis of 400 research projects reviewed by Medicinal Chemistry Study Sections A and B. A useful system for analysis of such data was established, tested, and with modification is now being continuously utilized by the executive secretaries of the Medicinal Chemistry Sections.

Interagency Activities—The ORAE has supplied the representative for the NIH to the Committee for Federal Representatives for Chemistry and has maintained close contact with the development of the NAS-NRC Report on Chemistry in the U.S. (Westheimer Committee on Chemistry). The NIH was represented by an ORAE staff member at the July 1965 Woods

Hole Summer Study meetings of the President's Scientific Advisory Committee during its review of the needs of chemistry. The Associate Chief for Research Analysis and Evaluation has continued to act as liaison representative for NIH with the NAS-NRC Committee on Chemistry.

The staff scientist who developed the ORAE Nutrition Report has been invited to attend the Nutrition Research Board meetings and to coordinate nutrition review studies from a variety of sources with this group.

Another staff member has represented the ORAE at the meetings of the Interagency Group for Research on Research, an informal association of research administrators from a variety of Federal agencies.

Other Activities—The ORAE has prepared numerous materials on NIH programs for use in reply to various individual requests by legislators. One example was the major contribution made by the ORAE to material extending the testimony of the Director, NIH, before the Subcommittee on Science, Research and Development of the Committee on Science and Astronautics of the House (Representative Daddario), concerning the role of the National Science Foundation.

Another ORAE activity has been the participation by a staff member in the studies of Task Force C of the Surgeon General's Advisory Committee on Urban Health.

Future Plans and Goals—Additional staffing and organizational changes are expected to continue during FY 1967. At least four additional scientists are needed to direct units on specialized fields of biomedicine in the Scientific Evaluation Section.

ORAE plans to organize other scientific meetings similar to the Bioengineering Workshop held in the Fall of 1965.

The Office will develop or adopt a disease classification which can be coordinated with the recently developed discipline and medical specialty codes in the central data file.

The ORAE has proposed that a central file of selected documents on PHS grants be established for analytical purposes in the DRG. Image reduction and filming techniques for storage are being fully explored. It is hoped that this file will be available and installed during FY 1967.

Studies on the utilization for analytical purposes of the grants list filed by central scientific classification codes are planned for the next year. ORAE is also cooperating with SAB in the DRG plans to automate and computerize the Research Grants Index file.

Policy and Procedure Office

The Policy and Procedure Office is responsible for assisting in formulating, promulgating, revising, and disseminating grant policy and procedure for NIH grant and award programs within the framework of PHS policies, and for initiating and conducting studies leading to formulating such policies on a PHS-wide basis. To this extent, the Office serves as a central repository to assure a unified and coordinated approach to developing, reviewing, and implementing PHS policies and procedures on grants and awards.

Detailed substantive reviews of extramural policies and procedures within the PHS are conducted to identify areas of omission or deficiency; new and modified policy for appropriate review and coordination is recommended; and problems and inconsistencies which proposed revisions in grant policies and procedures may create are anticipated.

The Office is also responsible for the administrative work incident to maintaining the Policy Statements and Guides to Operating Procedures for research and training projects.

Policy changes developed through PPO activities in the past year include indirect cost policies effected by the 1966 Appropriation Act; implemenation of policies and guidelines on cost-sharing in research grants; procedures for implementing PHS policy for all grants on the conduct of clinical research and investigation involving humans; and for protecting individual privacy in research investigation.

The Office is also responsible for preparing the Division's administrative report three times yearly for the study sections and other advisory groups.

Information Section

Data and information on all extramural grant programs of the PHS are provided to the public, the scientific community and program staff of the Service by the DRG Information Section.

The staff prepares press releases and other material for publication, answers public and press inquiries by mail and telephone, staffs PHS-DRG information exhibits at national scientific meetings, prepares reports on program developments, and maintains a reference room for the extramural staff.

During FY 1966, the Office continued to schedule appointments for visitors from grantee institutions seeking orientation in the policies and procedures of PHS extramural grant administration. Science writers and others were assisted by the staff in gathering facts and photographs for story material on the Service and the NIH.

The information exhibit, "Grant and Award Programs of the Public Health Service," was staffed by DRG information specialists at six scientific meetings during FY 1966. Designed and constructed to serve as a reception center where DRG scientist administrators

may meet and confer with representatives of grantee institutions, the exhibit carries on "across-the-board" collection of publications for distribution on PHS support of research, research training and construction of research facilities. Annually, the exhibit draws mass attention at the Federation of American Societies for Experimental Biology where more than 21,000 were registered for the Spring 1966 meeting held in Atlantic City.

The DRG Newsletter, edited and distributed by the Office to strengthen communications between PHS research grant administrators and grantees, became a monthly publication during FY 1966. The Newsletter was first issued in February 1964 to be published periodically to advise grantees of new and changing procedures in the administration of PHS grants and awards. The volume of response from grantee business administrators and investigators warranted more frequent editions of the Newsletter.

The DRG Reference Room serves the Westwood Building staff by adding current material to its accumulation of more than 8,000 pamphlets and 1,000 books on research accomplishments in the biomedical and health-related sciences.

Extramural Inventions Office

The Extramural Inventions Office (formerly the Extramural Patents Office) is responsible for obtaining and processing invention reports for all types of PHS grant and award programs. This function is performed in connection with NIH grants, as well as grants supported by the Environmental Health and Community Health Divisions of the Bureau of State Services.

During FY 1966, 236 new invention reports were received and 284 determinations and administrative closings were processed through NIH, PHS, and DHEW channels. For comparison, during the period from July 1, 1964, through April 15, 1965, 183 new invention reports were received. During this period, 154 determinations were sent to the Surgeon General for approval and signature.

Administrative responsibility relating to processing invention reports includes the following:

Reviewing and appraising invention reports resulting from PHS grants and awards;

Recommending to the Surgeon General appropriate determination on disposition of invention rights under DHEW regulations;

Interpreting PHS patent policies and procedures to PHS personnel, scientists, and officials in grantee institutions;

Acting in an advisory capacity to personnel in the grants branches of PHS institutes and divisions in carrying out patent policies and procedures; and

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Communicating with investigators and grantee institution officials, providing advice on responsibility for reporting inventions resulting from PHS support.

Grants Associates Program

The Grants Associates Program, established in 1961, develops selected scientists for administrative careers by providing insight and knowledge in the environment of federally supported research and the policy issues and administrative challenges implicit in the many programs of the PHS.

Further development of the program is scheduled to continue in FY 1967. Projected plans include a full complement of grants associates by the middle of the year with a modest increase predicted for the future.

The program is a unique educational experience in administrative skills and program analysis. For a 12-month period the associate participates in an individually structured curriculum developed through a preceptor approach according to his professional background and interests.

The training is tutorial in nature and PHS-inclusive in scope. The trainee is exposed to the current practices of grants administration, program analysis and grants management. Essential background information is obtained through reading assignments, by working with senior scientists within the PHS, visiting project sites, and participating in the evaluation and analysis of grant-supported research and in the planning of new programs.

During the year, a series of seminars is conducted for the associates. One entitled "Public and Science Administration" is presented to all associates and a selected number of extramural scientific staff from the NIH and other bureaus of the Service.

During FY 1966 this series was conducted by the American Society for Public Administration at Airlie House in Warrenton, Virginia. A published report of the seminar, containing an overall analysis of the discussions and a measure of effectiveness and general accomplishments will be made available to the participants. The Public Health Seminar and the Informal Seminar were combined into an afternoon session and presented as the Friday PHS Seminar Series, which included an informative discussion of problems and experiences pertaining to the associates' weekly assignments, and was followed by the seminar conducted by a senior scientist administrator from the PHS or a prominent non-Federal scientist or administrator.

Program Represents a Select Group—In this reporting period a committee was appointed to study current practices associated with the appointment of Grants Associates to the program. The committee recognized that the Grants Associates Program represents a select group of scientists and that the opportunity for setting standards may find a wide adoption throughout the PHS. The committee studied the current employment practices and formulated a number of recommended procedures.

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RESEARCH GRANTS REVIEW BRANCH

In FY 1966 several review functions, mentioned in "Highlights," were transferred to the Branch from other granting components of the PHS. This continues the trend of consolidating the scientific merit review of research project proposals in the Research Grants Review Branch.

Workload and program requirements resulted in the creation of the following study sections: Environmental Health and Engineering B, General Medicine B, Cell Biology B, and Biophysics and Biophysical Chemistry B.

The post of Conference Coordinator for the PHS was established within the Branch, and the duties of publishing the 18-month Calendar of National Meetings were transferred from the PHS Committee Management Office to the RGRB.

A sharp increase in the workload of the Referral Office and the Project Control Section is attributable to an increase in the number, size, and complexity of applications, and in the number and diversity of PHS granting components and special programs. The Branch processed and assigned more than 13,000 applications for new, renewal, and supplemental research grants (an increase of approximately 8 percent over 1965);1,100 for interim support; and more than 15,000 for continuation grants. In addition, 90 applications processed for the Joint Construction program and 121 for the Health Research Facilities program proved to be substantial contributors to the workload of the referral-control activity because of their size and complexity. In 1966, the HRF program was expanded to include grants for nursing facilities and will be further expanded to include grants for library facilities. Competing applications processed for the November councils outnumbered those for a single round of council meetings in the history of the grants program.

The large collective impact of several other programs on the workload of these activities promises to become even greater in the next year or two. Among these are the cerebrovascular projects, the health research facilities projects, the pharmacology-toxicology projects; child health program projects, the environmental health centers program projects, the exploratory research projects of the Heart-Cancer-Stroke program, and projects generated by the several panels of the U.S.-Japan Cooperative Medical Science program.

A permanent Special Programs Section was established in response to the increase in large program-oriented projects, and the increasing numbers of research proposals involving competencies which established study sections are not staffed to review. This Section coordinates and provides continuity and stability in the review of these proposals and in dealing with their special problems. It has greatly heightened the capability of the Branch to plan and initiate the review of applications for support under new programs. Examples of the programs thus undertaken are: cancer chemotherapy collaborative grants; program project centers in environmental sciences; clinical research centers in the field of cerebrovascular disease; out-patient research centers for the study of ophthalmological problems; and the six research areas of the U.S.-Japan Cooperative Medical Science program.

Evaluation and Stimulation Activities—The primary mission of each study section is to evaluate the scientific merit of research proposals. Since published data are available giving dollar amounts and approval rates, it will not be repeated in this report. However, the important secondary mission of each study section is to evaluate and stimulate the "state of the art," in its discipline. These established expert panels, representing the current spectrum of disciplines in biomedicine, present a unique resource for this vital activity. The sharp increase in this activity in 1966 represents an awareness by experts in these fields of a need to array and assess the gains, methods, and approaches; and to reset standards and goals.

This activity is usually accomplished through conferences and workshops sponsored or stimulated by a study section (sometimes in concert with other study sections) and by participation of study section members, in conferences sponsored by PHS granting components, professional societies, etc. Following are capsules of some of these activities in FY 1966:

Radiation Study Section - A 2-day Conference on Biological Effects of Lasers in October 1965 addressed itself to laser instrumentation, general biological effects of laser radiation, and the effects of laser radiation on specific tissues.

An agenda is being prepared for a conference between the Study Section members and the directors of existing Radio Therapy Training Grant programs. Topics to be explored include problems of trainee recruitment, didactic and clinical training program scheduling, integration of meaningful radiation biology and physics research programs, etc.

General Medicine A - A 3-day workshop on The Relationship of Mycoplasma to Rheumatoid Arthritis and Related Diseases, February 1966, cooperatively supported by the Bacteriology & Mycology A, Virology & Rickettsiology, Allergy & Immunology B, and Surgery B Study Section, and the National Institutes of Allergy and Infectious Diseases and Arthritis and Metabolic Diseases.

A workshop to plan a grant-supported conference on gastroenterology to be held in September 1966 included Study Section members, institute staff members, training grant committee members, representatives of professional societies, and university gastroenterologists.

Study Section members are interested in conducting developmental workshops in dermatology and in hepatorenal disease.

Dental Study Section - A 2-day workshop on the Biology of the Dental Pulp Organ in September 1965 was attended by 31 scientists. Several of the papers presented will soon be published.

A conference on Mechanisms of Salivary Secretion is planned for August 1966. Another conference on Craniofacial Growth and Development is planned for May 1967.

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A committee has met with the NIDR Training Committee to study and make recommendations on stimulating dental research.

Behavioral Sciences Study Section - A conference on Ethical Issues in Behavioral Research in June 1966 will deal with two major categories of behavioral problems: (a) The use of deception or coercion, either to secure the participation of the subjects or as an integral part of the research design, and (b) possible harm to subjects, ranging from the creation of anxiety states to the violation of assured anonymity. This conference is also expected to help crystallize the study section's guidelines for reviewing research proposals.

Pathology A Study Section - A workshop on Medical Genetics, presented by the University of Mississippi Medical Center, September 1965, and a seminar on Diagnostic Laboratory Techniques of the Communicable Disease Center was held in April 1966.

Genetics Study Section - A conference on Goals in Human Populations. This conference achieved an important clarification of divergent viewpoints of eminent investigators on the subject of the theoretical and practical objectives of population studies in human population genetics. A summary is being prepared for publication.

A workshop is being organized to evaluate and recommend upon the problem of exposure to chemical mutagens and the possibility of genetic damage to man.

History of Life Sciences Study Section - A conference on Biomedical Library Historical Sources, January 1966, established an informal exchange of survey information between archival specialists and librarians in a number of Government agencies.

A conference on Needs in the History of the Behavioral Sciences, April 1966, established guidelines for future workshops for researchers and teachers in the history of behavioral sciences.

In the summer of 1965, the Study Section established criteria for and assisted in a survey of teaching, training, and research in medical history in American medical schools.

Mental Health A Study Section - Symposia and workshops are contemplated in such areas as criteria of creativity, the definition and measurement or assessment of ego-functions, and specific problems in research with schizophrenic subjects.

Pharmacology & Experimental Therapeutics A Study Section - Study Section members are serving on an ad hoc panel, sponsored by the NIGMS on stimulating research in the area of comparative biochemical, pharmaco-dynamic, pharmaco-therapeutic and toxicologic action of a chemical from species to species in animal and plants. A symposium is planned as a preliminary step to the development of a broad program in this area.

Communicative Sciences Study Section - Five members participated in a conference on Brain Mechanisms Underlying Speech and Language, November 1965, sponsored by the Advisory Council of the NINDB.

A Study Section member is chairman of the group surveying the accomplishments and needs in the area of hearing as part of the NINDB Advisory Council Subcommittee on Human Communication and Its Disorders. This group met three times in FY 1966 and expects to publish an annotated bibliography of research on all aspects of human communication.

Members of the Study Section performed as symposium coordinators and as chairmen of the session on diagnostic evaluation of the National Symposium on Deafness in Childhood, May 1966, which dealt with the pressing problem of rehabiliation of the deaf.

Accident Prevention Research - A workshop on Techniques in Accident Prevention Research in April 1966 dealt with research methods and techniques, as well as the broader aspects of accident problems. Proceedings will be published by UCLA, the sponsor.

Nutrition Study Section - The workshop, Nutritional Anthroprometry-Methods and Problems, in fall 1965, focused on somatic criteria of nutriture with special emphasis on child growth and body composition. Part I surveyed and critically analyzed methods of determining body composition in vivo. Part II examined selected samples of current research and selected problems needing attention.

In winter 1965, the Study Section participated in a workshop on nutritional, metabolic, and endocrinologic effects of high terrestrial altitude exposure.

A workshop is planned for September 1966 on Regulation of Enzyme Synthesis by Dietary Means.

Environmental Sciences & Engineering B Study Section - The permanent planning committee identified fruitful areas for symposia such as the biological and engineering aspects of mechanism of lung function; social economic and political aspects of air pollution; recent developments in automatic counting and sizing methods for air particulate, etc.

This Study Section continues to participate in the Working Group for the Standardization of Respiratory Survey Methods.

Environmental Sciences & Engineering A Study Section - A grant-supported conference on Graduate Opportunities in Environmental Health will be held in the summer or fall of 1966 to develop a method for acquainting teachers and promising students with the scope, needs, and intellectual challenges of a career in the environmental sciences. The conference was stimulated by the combined efforts of the ESEA and

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the Toxicology Study Sections.

A symposium on Chemical Analysis of Water in March 1966 dealt with the chemical aspects of research in the field of water supply, waste water, liminology, oceanography, and geochemistry. Proceedings will be published by the American Chemical Society.

Stimulated by the ESEA and the Toxicology Study Sections, a conference on The Use of Agricultural Chemicals was held in October 1964. The proceedings were published in the book, Research in Pesticides, September 1965, by Academic Press. The conference examined techniques assessing hazards of chemicals in food additives and reviewed and integrated the present status of knowledge in controversies arising from the use of pesticides, herbicides, and insecticides.

A workshop on Anerobic Waste Treatment, November 1965, summarized the work in biochemical degradation of waste and docused on pertinent problems.

The ESEA and TOX Study Sections also stimulated a 2-week workshop seminar in water chemistry to raise the level of understanding of modern water chemistry and chemical fundamentals of teachers and researchers in sanitary engineering.

A conference on Pollution and Marine Ecology in March 1966 identified the critical areas of research in acquatic biology, and focused on improving quality and productivity of research in this field. Proceedings will be published in book form by John Wiley Press. A subcommittee of the ESEA Study Section prepared a statement, "Manpower Resources in Environmental Sciences and Engineering," which was submitted to the Office of Resource Development, BSS(EH).

In March 1966, the ESEA Study Section met with representatives of Federal and private organizations in the water pollution field and gave impetus to the timely publication of water research in appropriate journals.

In April 1966, the ESEA Study Section stimulated and participated in a workshop seminar on the systems analysis of such problems as water resources, solid waste disposal, and treatment design.

Neurology A and B Study Sections - The visiting program in neuroanatomy developed by the Steering Committee and implemented in 1964 under NINDB grant support has proven to be a highly successful program and is expanding. The program is designed to attract students to neurology through neuroanatomy to increase the number of teachers and investigators in that field.

In concert with the NINDB, the NEUA and B Study Sections' Committee on Cerebral-Vascular Research held a 2-day workshop in January 1965, conducted by noted researchers in cerebral blood flow mechanisms. Basic considerations of rheology and its clinical applications were discussed. As a result, 24 new research proposals have

been received dealing with cerebral blood flow physiology.

The Neurology Study Sections gave impetus to a 2 1/2-day conference on The Invertebrate Nervous System in January 1966, sponsored by the NINDB and the California Institute of Technology. The papers presented will be published by the University of Chicago Press.

Tentative plans are being made for workshops designed to promote fulfillment of the pressing need for collaborative efforts between experts in neurology and those in bioengineering and statistics.

Plans are also being laid to link needed areas of research with the new Regional Medical Programs.

Toxicology Study Section - This Study Section has undertaken a project dealing with the toxicological aspects of food technology and is developing a symposium on food problems of the Western Hemisphere. The Program Planning Committee has also stimulated several research proposals in toxicology research; a symposium on inter-American functions of pure food supply; the publication of a book to serve as a compendium of food problems; and a 2-day conference on graduate opportunities in environmental health.

Biophysics and Biophysical Chemistry A Study Section - Under this Study Section's stimulus, inexpensive, accurate atomic chemical models have been developed and are ready for commerical distribution. They provide an important new tool in biochemical teaching, training, and research, and will assist scientists in visualizing the complex 3-dimensional architecture of key body components such as those of the enzymes and genes.

A conference on mass spectrometry in 1965 created explosive interest in this important biophysical chemical tool. As a result, two United States leaders in the field greatly strengthened their research and training facilities and several applications for support of research and training in this field are expected in the near future.

Endocrinology Study Section - A workshop is being planned for FY 1967 dealing with the mathematical analysis of data in experimental endocrinology.

A subcommittee on standards continues to hold meetings to encourage the establishment of uniform assays and standards. Under the Study Section's Hormone Distribution Program, the human growth hormone, follicle-stimulating hormone, luteinizing hormone, thyroid-stimulating hormone, prolactin and growth hormone have been prepared in highly active form and distributed to research establishments throughout the world. The number of requests for pituitary hormones has greatly increased to nearly 1,000 in 1966. Relaxin is being assayed for standardization and when completed will be available to this Study Section for distribution to qualified investigators. The preparation of parathormone remains a subject of concern.

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STUDY SECTION MEETING—Study section members meet three times annually to give initial review and scientific evaluation to research grant applications. The study section's executive secretary and his grants assistant are shown seated at the far end of the table with section members who discuss the scientific merit of a research proposal and assess research needs in their respective areas before voting a priority rating for each proposal. Study sections average 15 members, all of whom are competent scientists selected from research institutions and universities throughout the United States. The study sections function administratively within the Division of Research Grants, each with a chairman chosen from the consultant membership, and the nonvoting DRG staff scientist who serves as executive secretary.

Tropical Medicine and Parasitology Study Section - A workshop in April 1966 identified certain neglected areas of research, principally epidemiology and clinical research needs in tropical medicine; significant opportunities in medical entomology and ecology; stimulation of interdisciplinary research plans and planning for optimum rapid followup of leads in laboratory and field investiations; and the international biological productivity.

In April 1966, the Study Section participated in the program of the Tropical Medicine Association of Washington, D.C., dealing with the present-day medical problems of Southeast Asia.

Surgery A and B Study Sections - The Surgery Study Sections jointly stimulated a conference on Clinical Transplants and Related Problems to be held in January 1967. This conference is expected to survey progress and identify major basic and technical problems in this rapidly expanding field; disclose the prospects and pitfalls of the various approaches to particular aspects of the problems and possible future directions; yield, through its discussions and published proceedings, increased coordination and effectiveness of research efforts in this field and qualitatively upgraded research grant applications; and improve and enhance the knowledge and effectiveness of study section review methods.

Pathology B Study Section - This Study Section is concerned with the training of medical students in the field of pathology, and is proceeding with plans to formulate suggested curricula which will provide minimum training for the production of clinical pathologists.

The widespread gaps in course content include the growing body of material dealing with cellular and subcellular aspects of disease, an omission which continues to widen the gulf between molecular biology and pathology.

Medicinal Chemistry A Study Section - This Study Section is actively engaged in a project to find and suggest a solution to the problem of making adequate testing facilities available to chemists for screening the products of their research.

Trends—Research proposals in the area of physical pharmacy are gaining greater importance in their relationship to drug therapy. Study section competence for this review has been accomplished by a joint review of the Pharmacology & Experimental Therapeutics A and Medicinal Chemistry A Study Sections.

A recent marked rise in research proposals dealing with respiratory research reflects increasing interest possibly connected with heightened public attention to air pollution and related problems.

The Hematology Study Section receives an increasing number of proposals directed toward the area of immunohematology, probably reflecting the development of ion exchange resins and gel electrophoresis techniques of recent years.

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The Radiation Study Section reports an increase in grant applications involving biological applications of laser light.

Dermatology applications in the General Medicine A Study Section doubled in 1966.

Dramatic strides in the study of ultrastructure and the progressive rise of technical standards in electron microscopy have prompted the Pathology A Study Section to heighten the qualitative review criteria for proposals submitted by electron microscopists.

The Genetics Study Section reports that studies of human populations have been receiving increased emphasis in recent years. Of particular interest to geneticists are selective factors which maintain polymorphisms, genetic loads and effects of inbreeding, and genetic drift in small populations. Recent work on the induction of mutations by chemical agents has shown that a considerable number of compounds are powerful mutagens. Some of these are exceedingly mutagenic for microorganisms without being toxic to man. The possibility that certain, relatively nontoxic substances might be highly mutagenic to man is a matter of considerable concern to geneticists.

In FY 1966, a definite trend emerged toward histocompatability typing of leucocytes, spurred primarily by two influences: (1) Immunosuppressive drugs used in clinical transplants are more effective in prolonging graft survival when there is increased histocompatability between the donor and recipient, and (2) the techniques for studying leucocyte antigens have been developed to the point where such studies are not only feasible but have a much higher chance for success.

The Endocrinology Study Section reports that programs involving the preparation and distribution of pituitary hormones and radioactively labeled steroids have increased in 1966. The National Pituitary Agency, which was initiated through the efforts of this Study Section, is currently collecting 75,000 glands yearly.

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STATISTICS AND ANALYSIS BRANCH

Reorganization of the Statistics and Analysis Branch from four sections into three was approved by the Division Chief in August 1965. Another organizational change brought the functions and most of the personnel of the small Analysis and Evaluation Section of the Career Development Review Branch into the new Statistical Analysis and Surveys Section. Branch activities during FY 1966 have been supervised through the three sections. The following is a summary of accomplishments, current work, and plans for each of the three sections.

Data Processing Section

The Data Processing Section has made its major manpower investments in the improvement of the central data files, the design and implementation of several major additions to the Central Data System, and the redesign of the entire system for conversion to new IBM computer equipment.

During the first full year of computerized awards and continued grants review cycle materials preparation, the DPS has significantly increased the accuracy of keeping data current in the Central Data System. Consequently, a variety of timely and relevant reports are now being provided from the central data files routinely and upon request. The Section has increased the speed of response and tailored the data content to institute and division needs.

As training grant review cycle materials are computerized, the DPS will be able to provide reports on a widening universe of NIH activities.

Conversion to New Equipment—During late FY 1965, the DPS contributed to a review of NIH computer needs, which resulted in an NIH decision to acquire a new large-scale IBM computer and to contract for the conversion, by IBM, of existing computer programs.

This decision has provided the DPS with the opportunity to spearhead a joint DRG/IBM/DCRT redesign of the DRG Data Processing System. A 4-month systems study resulted in a design which incorporates the latest concepts of computer systems and makes optimum use of the new computer's random access storage and remote unit capabilities. The design also capitalizes on past experience to improve the efficiency and speed with which the DPS can maintain the central data files and produce both recurring reports and answers to one-time queries.

Implementation of this design has involved some 25 IBM programmers and extensive efforts by members of the DPS. Every major element of the DPS central data files and their interrelationships has been reviewed in detail and adapted to random access storage. Every DPS reporting system has been redesigned in detail. The power of the query system has been greatly increased to allow extensive and complex reports to be produced within 24 hours. The DPS programming and operating personnel have been schooled in three new computer languages and new operating concepts relative to large IBM computers.

The system is designed to allow integration with other NIH data files as well as later additions of remote units in the Westwood Building to communicate at maximum speed with these central data files through the use of the computers on the NIH campus. Capabilities for expansion are being sought with little sacrifice of standing commitments and necessary systems improvements. The conversion is scheduled for completion in February 1967.

Systems Development—Training Grants: The DPS began in December 1965 to capture information for its new computer-based system for training grants. By February 1966, over 5,000 competing and noncompeting applications for training support had been recorded. In March, the necessary computer programs were ready to produce Notices of Grants Awarded and Approval Lists as a service to the awarding institutes and divisions. Generally, the same type of information that is currently maintained for the research grant programs is now maintained for the training grant programs. Products include computer preparation of various informational listings and reports during the council review cycle; approval lists and award statements at the time of award; and listings of grants by State, grantee institution, fiscal year, etc., after award notices have been issued.

NIH Central Scientific Classification System: The DPS integrated the NIH Central Scientific Classification System into the Central Data System. In cooperation with the Branch's Research Documentation Section, the DPS has established means of classifying and coding every research grant application—beginning with the June 1966 Councils—according to this scientific classification system. The process of classifying depends on the institute or division concerned to exercise final judgment on the particular classification. The DPS has computerized this classification to allow quick turn-around query reports on research grants or applications with a specified classification or set of classifications.

Trainee Appointments: A new computer-based information storage and retrieval system was designed and developed to process information on trainees supported under PHS research training grants and general research support grants. Over 35,000 records of appointments made each fiscal year are expected to be recorded under the new system. Each record will contain 55 items of information, including the educational background of the trainee and the training sought, captured from the Statement of Appointment of Trainee (PHS Form 2271). A series of complex edits, data dependency checks, and calculations is being performed by the computer to insure accuracy in the data reported to and by the DPS. Data on trainees supported by FY 1965 funds are being captured, and computer reporting is anticipated early in FY 1967.

Change Notice Form (Form NIH 901): To maintain current and accurate Central Data System records, a uniform procedure and a standard form have been established to communicate changes between institutes and divisions and the DPS on items affecting records. This was necessary to reduce ambiguity in communicating changes, shorten the time in reflecting changes in the Central Data files, and establish a control on changes. The procedure calls for completion of a standard form (Grant/Application Change Notice—NIH 901) by the institutes and divisions as changes occur in their grant records. The Grant/Application Change Notice provides for the following:

(1) Direct communication between the institutes and divisions and the Central Data System of changes in grant records as the changes occur.

(2) Assignment of a control number by the institutes and divisions to each completed change notice. The control numbers are assigned in serial number order and will be checked by the DPS to insure that all transmittals are received.

(3) Complete identification of grant record affected to insure that the correct record is updated.

(4) Standard terminology and nomenclature.

(5) Documentation of changes as they occur. This should prove invaluable when attempts are made to reconcile institute records with Central Data System records.

(6) Simultaneous notification of changes in grant records to other affected points, by sending carbon copies of the original change notice.

Cost-sharing in Research Grants: To accommodate requirements for cost-sharing for FY 1966 and to provide annual reports to the Bureau of the Budget, major changes were incorported in the Central Data System. The modifications required extensive design and programming changes to the file maintenance and report computer programs. The Central Data System now enables the institutes and divisions to calculate grantee dollar contribution and percentage of PHS contribution on each award issued after March 1, and assures that appropriate calculations have been made on the applicable cost-sharing agreement. A monthly report for each grantee, listing all new or renewal grants and the percentage of contribution, will be provided to the PHS institutes and divisions. This information will be useful when a determination is made concerning the adequacy of cost-sharing on a grant.

Reports—The DPS improved its ability to produce a variety of recurring and nonrecurring reports. The Section's capability for producing reports oriented to grantee institutions and their geographic locations was significantly enhanced by a study of grantee institutions.

Organization of Grantee Institutions: The organizational structures of approximately 100 institutions, which collectively receive some 77 percent of NIH grant funds, were charted and described in detail in a publication, Organizational Charts and Comments for Selected Grantee Institutions. The DPS distributed this extensive study to segments of the NIH and the BSS, and to various other Federal agencies. Study findings have resulted in significant improvements in DPS reports on the 100 institutions. The DPS is extending this study to cover 56 additional institutions which receive $1 million or more in NIH awards.

Classification of Institution: To facilitate response to a growing number of requests from within the DHEW, from Congressional committees, and from Federal agencies such as the National Science Foundation, for information on grants to specified types of institutions (research institutes, institutions of higher education, etc.), a classification scheme

for coding NIH grantee institutions by type has been developed.

Reports of Active and Fiscal Year Awards to Date: Listings of all active and fiscal year awards to date by institute and division, State, city, and institution are being provided monthly and quarterly by the DPS to the PHS Regional Offices, to the DHEW, and to various NIH and other PHS components. These listings are used for program analysis, administration of Title VI of the 1964 Civil Rights Act, grants management, budget formulation and justification, and publications. The NCI and the NINDB use these reports bimonthly and quarterly for their official publications. Other institutes are planning similar applications.

Research Grant Applications Review Cycle Reports: During the first full year of computerized awards processing, the DPS produced thousands of application review documents and hundreds of reports on the pending applications. The increasing demand for these reports by NIH institutes and divisions and the consistent use of application review documents continues to demonstrate the integral relationship this data processing system has with NIH operations.

Query Reports: Since July 1, 1965, approximately 400 query reports have been produced by the DPS to answer requests. These reports are provided, in most cases, within 24 hours of the request. The query computer programs allow grant records in the central data files to be selected, sorted, summarized, and presented as specified by the user. The power of the query has been increased during FY 1966 to allow a greater variety of reports to be produced in the 24-hour turn-around period.

A large number of query reports have also been produced within the DPS for file maintenance needs. This mechanism has rapidly become the primary means of establishing close systems integration between the DPS and the institutes and divisions, and is leading to a greatly expanded reporting role for the Section.

Continuing Reports: The DPS continued to produce numerous recurring reports. The annual 5-volume PHS Grants and Awards, the Research Career Program Annual Report, the Monthly News Release, and the revised and expanded "26 T" report, Research Grant Applications Reviewed by PHS Study Sections and Committees, were produced and published. A number of less extensive recurring reports were produced along with several hundred nonrecurring reports on research and training data.

Congressional Reports: During FY 1966, the DPS received and responded to approximately 160 Congressional requests for information. These reports range from short, single-page tables to voluminous presentations.

Research Documentation Section

Research Grants Index—In August 1965, the fourth annual Research Grants Index (PHS Pub. 925) was published. The fifth edition (2 vols., 2432 pp) will be published in FY 1966. It will describe the essential scientific topics of over 17,000 research grants and contracts

supported by the PHS during FY 1965. The Index will be distributed to administrative personnel in the Federal government and to grantee institutions to disseminate information about current PHS activities in biomedical research.

Volume I (1,282 pp) indexes about 125,000 brief scientific descriptions of various aspects of the research projects under nearly 5,600 alphabetically arranged subject headings. Volume II contains three appendices.

Appendix I lists the research projects in order by grant number, name of investigator and his institution, title of research project, and cites recent articles published by the investigator. Appendix II is an arrangement of the research projects by broad research areas. Appendix III is an alphabetical listing of principal investigators.

Extractions and Compilations From the Index—The RDS prepares annually the compilations extracted from or ancillary to the Index. Two publications were extracted from the FY 1964 Index. One is a subject matter index to research grants awarded to scientists in foreign institutions. The other is an index to the research projects administered by the NIGMS. Similar publications will be made from the FY 1965 Index.

In November 1965, a preprint of the FY 1965 Index was published as an addendum to the FY 1964 volume. The preprint, which contains information about the new grants awarded during the year, is issued annually for administrative use to update the most recent Index while the revised edition is in prepublication stage.

A Statistical Summary of the Research Grants Index was produced by computer for the use of administrators of PHS science programs. It lists the terms appearing in the FY 1965 Index and the frequency with which those terms are associated with projects administered by the institutes and divisions.

Another summary, Statistical Data on Source of Publications Cited in the Research Grants Index, Fiscal Year 1965, is being prepared for the use of PHS science administrators. Part I of this summary will list 2,600 scientific journals or other sources in which PHS investigators published articles cited in the FY 1965 Index. Part II will show the journals according to total number of grantee scientists' publications.

New this year is a 2-volume Cumulative Bibliography giving the names, addresses, project titles, and publications of PHS-supported investigators for the period 1961-63. The bibliography is shown in grant number arrangement. A cumulative bibliography for the period 1961-65 will be published in separate volumes according to PHS institute or division administering the grants. Thereafter, the bibliography will be published yearly with cumulative bibliographies at subsequent 5-year intervals.

The Intramural Index, Fiscal Year 1965, was issued in March 1966. This subject-matter index of NIH projects is similar to the extramural Research Grants Index and is

published annually for use within the PHS.

The Section supplied specialized vocabularies from its Medical and Health Related Sciences Thesaurus (PHS Pub. 1031) to several Government agencies and professional societies interested in developing vocabularies in specific areas of biomedical research. Also available are computer print-outs of vocabulary terms used in the FY 1965 Research Grants Index. Separate vocabulary lists will be made for the NIH institutes and divisions.

Searches—In FY 1966, a punched-card deck was duplicated from the photocamera cards used for preparation of the FY 1965 Research Grants Index. The extra deck was made to facilitate answering special requests for information. Last year the Section made approximately 20 searches a month for scientists, administrators of scientific research programs in Government, Congressional offices, and professional organizations.

Plans for a Computer Operation—At the request of the DRG, the Computation and Data Processing Branch of the DCRT made a study of the manual procedures involved in the production of the Research Grants Index. The study was done in connection with the preparation of a request for a proposal to transfer the Index operations to computer.

NIH Central Scientific Classification System for Research Grants—Professional staff of the Research Documentation Section analyzed and coded all NIH research grant applications submitted for June 1966 Council review, according to the NIH Central Scientific Classification System. The coding sheets for each application were transmitted to all NIH institutes and divisions for review. Computer processing of the coding was performed by the Data Processing Section, SAB.

Statistical Analysis and Surveys Section

The Statistical Analysis and Surveys Section was created in August 1965. The new section was formed from the Operating Statistics Section and components of the Special Surveys Section. Later, in November 1965, the Analysis and Evaluation Section, CDRB, was transferred to the SASS.

The SASS has four functional units which are discussed below.

Forecasts and Projections—Plans were initiated in FY 1965 for utilizing the Central Data System to make projections of current year and first and second future year research grant financial requirements for each NIH institute. The purpose was to aid the institutes and divisions and the Financial Management Branch in formulating budgets and studying budgetary trends and needs.

In FY 1966, the planning stage was completed and several comprehensive projections were prepared. Studies were made of the methodology to be employed, the kinds of rates to be used, and the ability of the Central Data System to provide the required primary data. Trial and error experimentations were conducted to test the results of the projection to develop statistically reliable projection techniques.

The first projections, prepared in October 1965, included (1) FY 1966 research grant noncompeting continuation awards, competing renewal grants, and supplements to noncompeting continuation awards and competing renewal grants; and (2) FY 1967 research grant noncompeting continuation commitments and awards thereon and supplements to noncompeting continuation awards. These were followed by similar projections in January 1966 for the regular program only. In December 1965, an analysis was completed entitled: "A Study of Rates Used by DRG to Project NIH Research Grant Budget Requirements by Institute for Fiscal Years 1966 and 1967."

In March 1966, projections were completed for FY 1968 noncompeting continuation commitments and awards, carrying the October projections forward one more year. The October projections were also partially revised to reflect the FY 1966 supplemental appropriations for heart disease, cancer, and stroke and the FY 1967 President's budget.

The projections for all years were later updated, primarily by applying revised rates prepared from current data. The projection techniques provide for such timely revisions and updatings as new information becomes available. Revised projections and an analysis of projection techniques, rates, and procedures were consolidated and distributed as a single report in April 1966.

Objectives for FY 1967 include continuing efforts to improve the projection techniques, to refine and maintain the rate structure, and to update and increase the accuracy of the data base; extension of the projection period to FY 1969; and expansion into other areas, such as research grants competing renewals and training grants, to meet anticipated and expressed requirements of the OD, NIH, and the Comptroller's Office, DHEW.

Statistical Surveys and Sampling—A sample of 2,280 research projects supported with FY 1964 funds was selected, and detailed information was coded from reports of expenditures for those projects. A report was prepared for expenditures on research grants awarded from FY 1964 funds, and comparisons were made with comparable data for FY 1960.

A sample of FY 1965 research grant awards was also selected and a study was made of the first-time principal investigators who received an FY 1965 award. These data were compared with data for FY 1960 and a report was prepared.

A series of reports will be prepared during FY 1967 on items from the selected samples concerning information which is not routinely captured as part of the Central Data System, such as a comparison between the budget and expenditures, principal investigators' degrees, place of birth, etc.

Planning for the first survey will be completed by the end of FY 1966. It will be carried out in FY 1967 and will have as its general objective the determination of amount of support given to graduate students, as employees, on NIH research grants.

In FY 1966, SASS became involved in a study being conducted by the Office of the Secretary concerned with the operation and administration of research grants within DHEW

grantee institutions. The Section is providing statistical consultation and is selecting samples of institutions and grants to be evaluated. The Section will also participate in reviewing questionnaires and tabulating selected data.

Analysis and Evaluation—A Study of the Distribution of NIH Extramural Program Funds to the States, Fiscal Years 1955-1965 explores the distribution of funds from each of its six major programs to the States over a 10-year span, with particular emphasis on the years 1955, 1960, and 1965. In addition to reviewing the distribution of gross funds and per capita funds from the programs to the States, the study reviews fund distribution to various indices related to resources in the States. These indices, in addition to population, include scientists, physicians, graduate enrollment, and personal income. The review of research grant and training grant applications at council and institute levels is analyzed on the basis of State of origin. The report was released in April 1966.

The Section also studied the distribution of PHS FY 1965 funds by program to various institutions within the United States. The study is structured along geographic lines, concentrating on some 50 standard metropolitan statistical areas as defined by the Bureau of the Census. Areas with at least one medical school were selected. Detailed analysis is confined to funds of medical schools and hospitals or medical centers affiliated with these medical schools in the 50 areas studied. PHS funds to other institutions within the areas studied are treated as a whole. The study was designed to furnish data useful to the new Division of Regional Medical Programs for review of resources to institutions through the PHS extramural programs. Funds directed to clinical research on heart, cancer, or stroke are determined.

In addition to the two studies, five papers relative to the grant programs were prepared. The first paper presents data on the number of research grants by size in FY 1965, indicating the proportion of larger and smaller grants ($100,000 and over; or under $100,000) by program.

The second traces the distribution of NIH research grants by size of grant and funds awarded by each institute in FY's 1954, 1962, and 1965.

The third reviews the funding pattern of program-project (PO1) and categorical clinical research center project (PO2) grants active in FY 1965.

The fourth compares FY 1965 funds awarded with FY 1966 funds committed for research grants active during FY 1965.

The fifth reviews the initial review group actions for competing research grant applications by size of requested amount.

At least four major reports will be prepared on studies to be conducted during FY 1967, in addition to several smaller papers on various aspects of the NIH and PHS programs.

Operating Statistics and Special Reports—The activities of SASS in this area are summarized

in three groups: recurring reports, special reports, and internal reports and analyses.

Recurring Reports—

Basic Data Relating to the NIH: Published annually by the NIH, this pocket-sized reference book presents information on programs and resources of the NIH. Data for the publication are collected, compiled, and edited by the SASS. Continuing review and evaluation of the publication's contents will result in modifications to make the book more useful.

National Science Foundation Federal Funds for Science Survey: Data for the NIH portion of the survey are compiled annually for the NSF. The NIH reports a variety of data on its research activities by administering institution and performer, field of science, geographic area, basic research activity, applied research activity, etc.

Reports on Research Grant Applications Received for Review: Two reports have been distributed to each of the institutes and divisions, and to the FMB, OD, approximately four times during each council period. Reports are prepared 35 to 45 days after research grant application deadlines and biweekly until the initial review group meetings are over.

FY 1966-70 Research Grant Commitments Based on Grants Active July 1, 1965: The SASS prepared a series of tables on research grant commitments for each of the NIH institutes and the BSS divisions for the Comptroller, DHEW. The data are broken down by current type of grant for both the "regular" and "special" research grant programs. The commitment data obtained from the Central Data System for FY 1966 and FY 1967 were reconciled with the institutes and divisions.

Summary of Initial Review Group Actions on Research Grant Applications: This report provides the institutes and divisions with current data concerning the initial review group actions on competing applications to be reviewed by their respective institute or division advisory councils. These reports were prepared four times during each council period,twice during the initial review group meetings, once immediately prior to the first advisory council meeting, and once after all the initial review group data had been recorded.

PHS Report on Obligations to Institutions of Higher Education—Fiscal Years 1963-1965: These data were prepared for the Task Force on Statistics of the FCST Committee on Academic Science and Engineering for use as a basis for a report to the President on "Federal Obligations to Institutions of Higher Education." Decks of punched cards were supplied to the DHEW Coordinator for the Interagency Task Force. Data contained in the cards for each institution consisted of NIH and BSS-EH funds for support of research projects through grants and contracts, support of research resources, funds for research and development plant, training grant funds, fellowship funds, and general research support grants. Approximately 125 man-days were required to comply with this request. Specifications for computer programs to run this report in the future have been prepared by the Section.

Status of Programs and Resources of the NIH: This handbook consists of approximately 60 tables, charts, and graphs which depict the trend and current status of NIH programs

and resources. Tables and charts are updated periodically, some monthly, others annually. Additional charts and tables will be added to this handbook as more data are generated. Selected data will be taken from this handbook and distributed within the NIH and the PHS with appropriate analyses.

Summary Table for Grants-In-Aid Publications: The Section assisted in preparing in FY 1965 summary for financial assistance programs of the PHS. Data represent apportionments by program and institute for FY 1965 and FY 1966.

Additional anticipated recurring reports prepared for the institutes and divisions will double during the next fiscal year with the addition of the training grant data to the Central Data System. Specifications are now being drawn up to obtain summaries of competing training grant applications, as well as initial review group and council actions.

Special Reports—Requests for statistical data from members of Congress, DHEW, the Office of the Surgeon General, etc., were coordinated by the Section. Examples of these requests are for distributions of initial review group priority score recommendations (a request of the Fountain Committee); actions on competing research grant applications reviewed during Council Year 1965 with start dates during FY 1965 (requested by the Rogers Committee); and summary statistics on research grant applications, approved by council with FY 1965 requested start dates but not funded (requested by the OD for budget hearings).

Approximately 400 requests were received from the institutes and divisions for information which was obtained via the query system. Requests for special data from the Central Data System are coordinated by the SASS.

Internal Reports and Analyses—Many requests for information on intra- or extramural programs come from management within the NIH. Such requests may be for data concerning some program or activity where previous information is not generally available, or an evaluation of the number of research grants coming into DRG for assignment after a deadline.

During FY 1966, the SASS provided statistical services to the DPS, SAB, for Part V of the PHS Grants and Awards for FY 1964, and to the Office of Research Analysis and Evaluation, OC, concerning the geographic distribution of NIH funds.

CAREER DEVELOPMENT REVIEW BRANCH

Interest in the Public Health Service research training programs coordinated by the Career Development Review Branch has continued to increase.

Fellowships Program

Review and Advisory Groups—The Central Qualifications Board review groups are no longer referred to as "panels" but as "fellowships review committees."

Because the number of applications received each year has been consistently larger, it was necessary in FY 1966 to increase the former 12 committees to 15 by dividing Behavioral Sciences, Biochemistry and Nutrition, and Medicinal and Organic Chemistry into A and B groups. This also required the appointment of additional non-Government consultants. The additional committees, professional staff, and committee members gave impetus to the preparation and subsequent publication of a manual entitled Guidelines for Assignment to the CDRB Fellowships Review Committees and Roster of Committee Members.

A Fellowships Assignment Advisory Committee composed of three executive secretaries of the review committees has been established in the CDRB. This group has the final authority for the referral of applications which have relevance to two or more of the Fellowships Review Committees. Fellowship applications are now being routinely assigned to the Divisions of Accident Prevention and Community Health Services, BSS, and to the National Library of Medicine.

Revision of the Fellowship Policy Brochure—Several changes, including an appendix of all extramural forms used in the fellowships program, have been incorporated in the revision of the policy brochure dated July 1, 1963. The new publication, entitled Research Fellowships Program Administrative Guide, was developed for use by all PHS personnel concerned, as well as university deans, sponsors, and coordinating officials.

Improvement in Forms and Documents—Four-page announcements for each of the three types of fellowships were developed. Basic information such as eligibility requirements, stipends, supply and travel allowances, tenure, and method of reviewing applications has been included. A mass mailing of 15,000 of each of the announcements was made to the universities to stimulate interest in the programs.

Three booklets setting forth the applicable policies and procedures for each type of fellow (predoctoral, postdoctoral, and special) were published to serve as a reference during the fellowship tenure. These are mailed to an awardee with his Notice of Research Fellowship Award.

A new form, "Notification of Final Action on Fellowship Award," has been developed to expedite the processing changes in fellowship awards, such as increasing or decreasing stipends, dependency allowances, and tuition. The use of this internal form, in lieu of a

revised award statement and accompanying memo, should result in a saving of many man-hours and an efficient method by which institutes and divisions can convey information to the Financial Management Branch, OD, NIH.

Changes in Fellowship Regulations—The DHEW Secretary directed that the requirement of disclaimer of subversive affiliation be eliminated from the fellowship regulations while retaining the loyalty oath requirement. The CDRB staff provided assistance to the General Counsel's Office in developing the language of the regulatory change and handled the notification to PHS staff and fellowship coordinators.

The CDRB staff participated in developing and explaining Subpart A, particularly the proposed provisions for (1) hearings for fellows whose awards are determinated or whose applications for award continuation or renewal are denied on grounds relating to moral character or loyalty, and (2) restriction of sponsorship of fellows to nonprofit institutions.

Discussions were held with representatives of the Office of General Counsel and the National Library of Medicine to develop changes in Subpart A to cover NLM fellowships.

The CDRB maintained liaison with (1) the Personnel Management Branch, OD, NIH, on its development of Subpart B (service fellowships) inasmuch as both Subparts A and B are required to replace the existing fellowship regulations and (2) the OSG's Management Policy Branch to acquaint its staff with the interrelationship between the subparts and with the planned clearance by NLM with its Board of Regents.

Survey of PHS Special Fellowships—As a result of inquiries from several universities, the General Accounting Office, and others, a survey has been made of the practices of all PHS fellowship awarding units pertaining to special fellowships (manner of review, method of determining stipend, etc.). The findings will be correlated into a single report.

Stipend and Supplementation Survey—An extensive survey was made to obtain the opinions of both university and PHS members of the adequacy of current stipend levels, and the suitability of PHS policy on supplementation. It was proposed that, within certain limits, the matter of supplementation be left to sponsoring institutions. A final report was submitted to the Grants Policy Office, OSG, PHS, on February 28, 1966.

Information Exchange—The CDRB staff are developing a system which will inform other Government agencies and our embassies abroad of the presence of PHS fellows in foreign countries. This information can be used to set up various types of meetings of American citizens and in eliciting certain types of information from the fellows, e.g., the adequacy of laboratories and the competency of scientists.

Research Career Program

The moratorium on new research career awards remains in effect. Considerable effort is being expended to develop an award which would embody the original purposes of the

career award and, at the same time, eliminate the problems which caused the moratorium.

Development of Forms and Documents—The continuing evaluation of the career development award will undoubtedly result in program modifications. Although many projects are dependent on the outcome, others are progressing very well. The Policy Statement is being revised to incorporate information on civil rights and the moratorium on new career awards. The instructions for completing new, renewal, and continuation applications have been revised. This revision should assist in reducing the errors and omissions most frequently encountered in applications, and should prove beneficial to the applicant by presenting all instructions in one document.

A Research Career Program Reference Guide was developed and distributed to awarding unit personnel. The guide gathers all of the important aspects of the program into one book of policies, procedures, information, and statistical data, and is expected to serve effectively in communicating new program information to RCP awarding unit representatives.

During the first half of FY 1966, 549 career development applications were received, of which 156 were new, 56 were renewals, 270 were continuations, and 67 were supplementals. During the same period in FY 1965, 513 applications were received, of which 146 were new, 94 were renewals, 234 were continuations, and 39 were supplementals. A total of 100 career development applications received during FY 1966 consisted of 19 renewals, 71 continuations, and 10 supplementals, compared with 114 applications in FY 1965, consisting of 32 renewals, 75 continuations, and 7 supplementals.

There were no new career award applications because of the moratorium.

In summation, renewal applications decreased 41 percent, continuations increased 10 percent, new applications increased 10 percent, and supplementals increased 67 percent.

Training Grants Program

A total of 5,705 applications received and assigned to NIH institutes and divisions of the Bureau of State Services included 1,153 new applications, 791 competing renewals, 580 supplementals, and 3,181 continuations. This compares with a total of 2,878 applications received during the FY 1965 review period. Of the FY 1966 total, 462 were new, 393 were competing renewals, 336 were supplementals, and 1,687 were continuations.

The system of central receipt and assignment of training grant applications formerly sent directly to the National Institute of Mental Health has been effected. As a result, the assignment and processing workload increased 60 percent (2,374 additional applications) during the 6-month period in which these applications were received.

Mailing Index—A training coordinator mailing index was established to serve all PHS training programs in the dissemination of information concerning policy and program matters and to advise the institution of reporting requirements. The index includes academic institutions,

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hospitals and medical institutions, State health departments, private research organizations, and others.

Visa Symbols—The CDRB staff in conjunction with the Visa Office, Department of State, and the Immigration and Naturalization Service, has developed a complete list of current visa symbols. This list has proven to be a valuable aid in the appointment of noncitizens to the various training programs by institutions which are recipients of PHS training grants.

Developement of Forms and Documents—A handbook entitled Training Grant Programs of the Public Health Service has been developed. The handbook outlines the training programs offered by the various awarding units of the PHS and identifies those offering training grants, and includes information regarding the purpose, eligibility, types, and financial assistance characterizing each program.

Considerable time has been devoted to consultation with institutional officials on policy and procedural matters relating to training grants.

Revised application forms and instructions have been developed, approved by the Bureau of the Budget, and put into use.

Other Program Activities

PHS Support for Active-Duty Military Personnel—The question of the eligibility of active-duty military personnel for PHS fellowships and traineeships (direct and indirect) has arisen several times. In the absence of a PHS policy covering this question, a staff paper was prepared which proposed several alternative policies based on information obtained from contacts with representatives from several agencies. It is hoped that it will lead to a PHS policy release covering such eligibility.

Compilation of Reports—During the course of the year, many reports were compiled as a result of congressional and other requests.

The CDRB staff prepared a report on "Extramural Training Programs for Professional Personnel in Health Fields Supported by NIH" which was submitted through channels to the Rogers Subcommittee of the House Interstate and Foreign Commerce Committee.

A report was compiled and forwarded to the Deputy Grants Policy Officer, OGS, PHS, on the fellowship, graduate training grants, and research career programs. This information was to be used by the Committee on Academic Sciences and Engineering of the Federal Council on Science and Technology. A later report included data on extramural training programs that support prebaccalaureate training leading to the M.D. or similar professional degrees.

CDRB Team Visits to Universities—Team visits to universities were initiated in early FY 1966 to disseminate information on PHS programs and orient faculty and students. This approach makes it possible to cover a larger segment of the university and has been well received.

The Branch fulfilled a key role in developing, implementing, and coordinating PHS policies and procedures applicable to (1) the issuance of publications effective July 1, 1965, replacing previous policy statements governing research and training grants; (2) implementing revised indirect cost and cost-sharing policy and procedures; and (3) clarifying internal procedures governing the administration of project period grants.

Committee Assignments—The staff served on committees to develop and modify grant application forms and instructions to implement cost-sharing policy and the revised indirect cost policies.

The Branch cooperated in establishing working committees to develop internal operating procedural documents to provide more effective grant administration. The Branch also provided support to the Pilot Study Committee on increasing the role of grantee institutions in the management of research projects. GMB assisted the Committee in selecting institutions to participate in the study, and in assessing experience gained under the first year's operation.

Special Assignments—Three members of the senior staff were temporarily assigned to assist with implementing Title VI of the Civil Rights Act of 1964 and to assist the Division of Regional Medical Programs in formulating policies and procedures governing eligibility, awarding, and funding mechanisms.

The Branch was also assigned a major role in settling accounts involving payments for medical services provided for research patients in 62 general clinical research centers supported by PHS grants during fiscal years 1960 through 1964. Site visits were made to all grantee institutions involved to discuss acceptable methods for cost determination, and to obtain proposals for settlement of these accounts. Settlement of approximately 25 accounts was completed. Negotiations for settlement of the remaining accounts were in process at the end of the fiscal year.

Meetings—The Branch was represented at seven regional and national meetings of college and university business officers and participated in the programs to exchange information relative to PHS grant program policies and procedural requirements.

Direct and Indirect Cost Negotiations—During the year, rate negotiations were initiated with approximately 200 grantee institutions to reach initial or renewal agreements based upon the principal of reimbursement of costs for services provided for research patients. Rate negotiations were completed with approximately 155 grantee institutions during the fiscal year. Initial discussions had been undertaken, or negotiations were in progress, with the remaining institutions at the fiscal year's end.

With the revisions in the Service's indirect cost policy, the Branch took on additional pre-audit responsibilities for checking indirect costs claimed by grantee institutions.

Special Studies—An analysis was made of research grants awarded in fiscal year 1964 for

which reports of expenditure have not been received to determine rate of delinquency, and reasons for delinquency. Approximately 2,000 grants were reviewed. The report was distributed to awarding institutes and divisions in February 1966.

A committee made up of bureau representatives was established by the Chief of the Branch to evaluate the project period grant concept and make recommendations to improve present funding mechanisms. This was at the request of the Chairman of the Interbureau Advisory Committee for Extramural Programs, PHS.

INTERNAL OPERATIONS BRANCH

The Internal Operations Branch provides administrative services for Division personnel and furnishes program materials for all extramural programs of the Public Health Service.

Office of the Chief—The Office of the Chief provides overall supervision of the branch activities and maintains liaison with other divisions and institutes in addition to performing specific functions such as space planning, forms clearance control, records disposition, and chairmanship of the DRG Safety Committee.

During FY 1966, the Office planned, scheduled and supervised approximately 182 office moves involving over 550 individuals and an undetermined number of office rearrangements. The management technician (forms analyst) revised and received approval on approximately 11 extramural forms and instructions; 15 new forms were designed for use in the automatic data processing area and 8 administrative forms were eliminated. The Office prepared a draft for the Application and Instructions and Expenditure Report for the Regional Medical Program. Also during this period, approximately 5,500 consultants' vouchers were processed and forwarded to the various study section chairmen for payment.

Office Services Section—This Section performs a variety of functions and duties including procurement, property, supply, equipment repair and maintenance, and documents relating to these activities. Another function is assembling and mailing an average of 10,000 grant and award application folders and 5,000 miscellaneous packages monthly.

Special Services Section—This Section provides services such as general typing, and control of the Flexowriters used in general letter typing operations.

The Section's Flexowriter punch card filing system prepares printer's copy for the publication Report of Negotiated Hospital Rate Agreement. During FY 1966, approximately 1,000 jobs were accomplished on the Flexowriters, ranging from a programmed letter to 1,500 previously cut cards for printer's copy.

The Section specializes in typing summary sheets, project site visit reports, manuscripts, manuals, speeches, letters, memoranda, and other forms of documents, as well as proofreading typed documents. The staff also transcribe dictaphone tapes. The services of this Section have been extended to include NIH institutes and divisions, and the SG's Office.

Mail and Files Section—This Section receives daily and processes for delivery a volume of incoming mail addressed to the Division. It also maintains audit files for the nine NIH institutes, the 13 divisions of the Bureau of State Service, the Division of Research Facilities and Resources, and of course, the DRG.

An estimated 24,000 grant and award applications will have been received by the Section during FY 1967, including forms PHS-398, -2590, and -4687-1. Similarly, 10 copies each of 17,800 reprints resulting from PHS-supported research will have been received, checked for

appropriate study section, and distributed. Forty copies each of some 18,800 progress reports will have been check for appropriate study section, recorded as to date received and grant number, and distributed to the study sections. Progress reports accompany competing renewal applications.

Another function of the Section is to receive and record certified and registered mail. A total of 20,000 pieces is expected.

The Mail and Files Section also receives and records grant refund checks. A total of 800 is expected. Audit shelving has been extended by 10 percent to take care of filing requirements as active use of audit files by NIH, PHS, and GSA auditors has increased. Incoming mail has increased approximately 50 percent over last year.

Travel Section—This Section's function is to receive and process for payment travel vouchers from DRG personnel and from consultants serving on review committees and study sections. During FY 1966, over 7,000 travel vouchers were received and processed, involving an expenditure of over $1 3/4 million. The Section also offers assistance to Division travelers in plotting itineraries, preparing vouchers, and obtaining tickets and travel advances.

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DIVISION OF RESEARCH SERVICES

Report of Program Activities
July 1, 1965, through June 30, 1966

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U.S. PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

DIVISION OF RESEARCH SERVICES

Report of Program Activities
July 1, 1965, through June 30, 1966

A. Objectives

The Division of Research Services was established in 1956 to provide professional and technical support to the medical research programs of the National Institutes of Health. In April 1966 DRS celebrated its 10th anniversary, reflecting on a decade of supplying scientific, technical, and engineering services to sustain and further NIH research programs. By keeping informed of these Institute programs, DRS is able to plan its own efforts to meet the daily requirements of the Institutes. The organization, staffing, and functions of DRS are adjusted accordingly to maintain an effective program of centralized services.

B. Current Programs

The Division of Research Services comprises some 1,300 persons with almost 200 individual occupational skills and is composed of an Office of the Chief and seven branches: Environmental Services, Biomedical Engineering and Instrumentation, Laboratory Aids, Library, Medical Arts and Photography, Plant Engineering, and Research Facilities Planning.

The programs of the Division can be divided into two broad classifications:

1. Programs in direct support of research

    a. Design, development, and fabrication of instruments and materials
    b. Biomedical engineering of methods and systems
    c. Production of bacteriologic and tissue culture media
    d. Preparation of sterile glassware
    e. Production of genetically defined rodents and rabbits
    f. Quarantine and conditioning of dogs, cats, and primates
    g. Maintenance of farm animals
    h. Animal surgery and recovery
    i. Animal health services
    j. Still and motion picture photography
    k. Graphic arts services and exhibits design
    l. Medical illustration and model making
    m. Library services
    n. Foreign language translation

2. Programs related to NIH facilities and proper environment

    a. Engineering liaison in the design and construction of new NIH facilities

b. Development of improved facilities and equipment
c. Operation, maintenance, and repair of the NIH physical facilities
d. Professional engineering and craft services for alterations, improvements, and installations
e. Maintenance of the NIH grounds
f. Environmental services designed to maintain and improve the health of NIH patients and employees

C. Program Progress and Accomplishments

Construction

Construction activities continued to expand at NIH, with DRS providing engineering liaison in the design and construction of new facilities. The current program of the Research Facilities Planning Branch comprises over 20 major projects with a total estimated cost of more than 75,000,000 in the planning, design, or construction stage. Seven projects were under construction in FY 1966, and two of these were completed. Design for several other buildings is nearly complete, and these will move into the construction phase in FY 1967. The extension of the General Office Building (31C) is expected to begin in August 1966. Beneficial occupancy should be effected in the spring of 1968. Multilevel parking facilities for Building 31 and the NCI-NIMH/NINDB buildings are planned, and the programs of requirements are in official clearance channels. Progress beyond the tentative sketches for the Child Research Center (NIMH) has been stopped pending selection of a new site on the south side of the NIH reservation. An addition to Building 21 is being planned, and work on the program of requirements is in progress. Construction of the new Biologics Standards Building (29A) is well underway. Beneficial occupancy should be realized in December 1966. Another project underway is the NCI-NIMH/NINDB complex and cafeteria (Bldgs. 35, 36, and 37). A new Gerontology Building, being built in Baltimore, Md., is scheduled for occupancy in the fall of 1967.

The proposed new Library facility and the Cafeteria extension in the Clinical Center (Bldg. 10) have been combined as a single project. A construction contract was awarded in April 1966, and construction is scheduled for completion in August 1967.

In 1966 funds were appropriated to expand Clinical Center facilities by adding two floors to the existing Wing D. It is estimated that construction will be completed by May 1968.

The program of requirements for a Virus Isolation Facility for the National Cancer Institute was completed in September 1965. Design was completed in May 1966, and construction completion is scheduled for early 1968.

Repair and Improvement

The increase in workload for engineering services was the most significant development. Construction performed by private contractors increased about fourfold. Engineering and planning staffs assumed an additional heavy workload due to the major improvements and new building construction at NIH.

Studies were made during the year designed to improve the capacities of the incinerator plant and the steam generating plant. Estimated results of one study showed that the capacity of the steam generating plant will be exceeded during 1968. A study is being made to determine whether it is feasible for NIH to combine with other federal agencies in using a central incinerator. To meet the need for more steam, an additional boiler is to be installed as part of the overall utility updating.

Extension of the drought through the third year (1962-1965) has had an adverse effect on the NIH grounds. New construction and a shortage of maintenance personnel have not helped the problem. Steps are being taken to alleviate the situation.

NIH Animal Center

Phase I of a three-phase construction program was completed last year. This included a farm animal building, a kennel building, a central utilities building, and two residences. Construction on Phase IA is scheduled to start in the fall of 1966. This phase will include a general laboratory building, an animal behavior laboratory and a waterfowl habitat for the National Institute of Mental Health, a primate building for the Laboratory Aids Branch, DRS, additional utilities, and an incinerator. Phase II will include an agricultural management building, an animal production facility, a waste chemical disposal facility, a third residence, and necessary supporting utilities and grounds improvements. This phase is still in the early stages and has not yet been approved by the Bureau of the Budget.

Instrumentation Support

The growing importance of biomedical engineering and instrumentation became increasingly evident during the past year at NIH. This area of professional and technical support has been significantly expanded. The Biomedical Engineering and Instrumentation Branch has been reorganized for improved utilization of research and development potential. Also, "satellite" technical support units have been established, improved workload and financial reporting with extension of automatic data processing has been accomplished, and a vigorous professional and technical training program has been undertaken. Branch professionals have made extensive contributions to programs involving mechanization, automation, computation, physiological monitoring, artificial organs, surgical procedures, analytical instrumentation, and laser technology.

Technical service sections processed more than 13,000 assignments, and Branch engineers were involved with approximately 400 various research and development projects.

The improved effectiveness of support in this area to NIH medical research programs results directly from emphasis on the enhancement of the role of the professional physical scientists and engineers as collaborators and prime contributors in health research rather than as subordinate service staff.

Shop Service Improvements

In FY 1965, preliminary steps were taken to initiate a material pre-packaging program. The program is designed to reduce the lost time resulting from shop employees having to wait in line at the Shop Stores issue counter when drawing out repair parts and construction materials. During FY 1966 an implementation schedule was developed, the basic criteria were established, and several pilot jobs were processed under the procedures. Initially, the program is being limited to pre-selected shop jobs involving over 250 man-hours of station labor. Within the availability of storage space and other limitations, this program was scheduled to be in full operation by July 1, 1966.

Full operation of the tool control program, including the operation of several satellite toolrooms, was scheduled to be in effect by July 1, 1966. Major planning for this program was completed in FY 1965. The tool crib area was cleared, and a substantial start was made in inventorying the tools and equipment to be included in the program. Initial requirements for storage cabinets and shelving were determined, and the cabinets and shelves were ordered. A schedule for implementation of the program was developed.

Library Services

Further implementation of the internship, bibliographical services, automation, and workload reporting programs was achieved during FY 1966. Specifications and equipment lists were prepared for the new library. Tentative plans were developed for moving personnel, collection, and equipment to the new library annex without major interruptions in service. Strong emphasis was placed on staff training programs during the year. A total of 37,537 books and journals were circulated, representing a decrease of 442 volumes since last year. The decrease results from restricting the circulation of materials of which only one copy is owned by the Library. Also, during FY 1966, 4,666 volumes were loaned to other libraries and 9,736 volumes borrowed from other libraries. Translations loaned to other libraries totaled 4,465.

During the year, the Library's collection was strengthened by the addition of 5,247 new books and by the receipt of 831 books through gift and exchange. For FY 1966, the Library placed subscriptions to more than 2,000 journal titles and received an additional 800 as gifts or on exchange.

A proposal for a satellite library for the Division of Computer Research and Technology was prepared. It is expected that this proposal will serve as a prototype for future satellite libraries at NIH.

Medical Arts and Photography

Reorganization of these activities has resulted in improved services. Medical and general art services were sharpened by organizational and policy changes. A work performance record was set by the Drafting Unit. Unusual pieces of medical art were drawings of the developmental stages of four newly discovered simian malaria parasites, models of arthritically crippled hands and feet,

a model of the human eye, and a new complete rat cranial atlas. A 1966 exhibit has been requested for long-term showing in the Smithsonian Institution.

In photography, trials are being made using roll film instead of cut film for use with patient and other clinical and scientific photography. There is also a slight trend toward increasing use of 2" x 2" slides over the heavy $3\frac{1}{4}$" x 4" size. Requests for motion picture work increased, especially for films of the "research summary" variety. Filming in support of clinical studies and research projects has continued. Capability in animation has increased.

Environment Control

Though long-term projects in this area increased in FY 1966, the bulk of the work continued to be on a short-term basis, solving day-to-day problems of researchers and their co-workers. Greatest program progress was made in activities associated with control of biological hazards. Building 7 was evaluated with regard to its suitability for hazardous work, and a suite for hazardous work was developed for Building 6.

Clean air at NIH has been a major concern for the past decade. An investigation defining the scope of the problem was completed and is serving as the basis for decisions on required changes.

Factors associated with contamination in animal production colonies are becoming increasingly important. The Building 14 production areas were surveyed to provide methods for solving potential problems in existing facilities and to provide information for use in the design of new facilities.

The laminar air flow concept developed for industrial clean rooms appears to offer a method for improving control of biological air contamination at NIH. A mock-up operating room using the laminar flow type of ventilation system was constructed so that new ideas may be developed and evaluated.

Animal Production

The Division produced over one million laboratory rodents and rabbits in FY 1966. Many animals were bred to specific investigator requirements. Shifts in the production operation were closely geared throughout the year to research requirements. Approximately 90 percent of the intramural needs for rodents and rabbits were provided from NIH breeding colonies.

Germfree Animals

Germfree rats, mice, and guinea pigs are being produced in stainless steel and flexible plastic isolators. A careful monitoring program assures that these animals remain germfree. Limited attempts to develop a diet and system for raising cesarean-derived germfree hamsters have proved unsuccessful. This project is to be pursued because of the great potential value of germfree hamsters for virology research.

Pathogen-free rats and mice are provided through the breeding of cesarean-derived animals within a barrier system. Current methods of producing pathogen-free animals are critically appraised for their suitability to future operations at NIHAC. A program to provide pathogen-free guinea pigs is underway.

Fertile hens' eggs free of the Resistance Inducing Factor (RIF) are produced by maintaining a flock of chickens within the barrier system. The birds are housed in individual cages, bred by artificial insemination, and regularly monitored for the RIF agent.

Animal Health

The most significant problems in the animal production area during FY 1966 related to diseases associated with breaks in sanitation. For example, salmonellosis necessitated eradication of the Strain 13 guinea pig production colony, and the Flemish rabbit colony was almost decimated due to coccidiosis. Fortunately the genetic back-up colony of Strain 13 guinea pigs did not become infected and could be used to reestablish the production colony. Overall sanitary practices and the physical environment have been carefully reviewed with the assistance of the Environmental Services Branch, and corrective actions have been taken wherever feasible.

D. Problems

Construction

Again in FY 1966, one of the most serious problems in the design program was the slippage which occurs in estimating progress schedules. These extensions of the design postpone the construction and beneficial occupancy of the research facility or project. This, in turn, further delays the implementation of the research program for the client institute. As a result of these delays, greater construction costs are incurred due to the escalation.

An analysis of the reasons for these design delays has been made. Two major factors appear to be the causes. The first is an unrealistic (optimistic) time schedule for review of the design. DRS has encouraged the inclusion of more realistic review periods in the design contracts. The second factor is lack of personnel to properly review the design. Personnel staffing in the Design Division of the Public Buildings Service, GSA, has been reduced in the past year by losses due to retirement and resignation. Vacancies created by these losses have not been filled. The PBS has also experienced an increase in its workload. The problem pertaining to the delays has been brought to the attention of responsible GSA officials regularly and continually by representatives of DRS and RFPB. No relief is in sight.

One additional factor in the delays can be attributed to the review of other groups such as the Commission of Fine Arts, the National Capital Planning Commission, the Maryland-National Capital Park and Planning Commission, and others.

DIVISION OF RESEARCH SERVICES

Summary of Branch Activities July 1, 1965, through June 30, 1966

RESEARCH FACILITIES PLANNING BRANCH Howard M. Biggs
Branch Chief

I. SUMMARY

A significant increase in construction activity on the NIH Reservation accurately reflects the progress being made in the current NIH facilities expansion program. The present Research Facilities Planning Branch program comprises over 20 major projects having a total estimated cost in excess of $75,000,000 in the planning, design, or construction stage. During FY 1966 seven projects were under construction, two of which were completed. Design for several other buildings is nearly complete, and these will move into the construction phase in FY 1967. Development of design guidelines, use criteria, and materials evaluations in areas such as animal caging, distilled water piping systems, duct materials, and floor and wall coverings provided valuable support and assistance to the design and construction programs of both the NIH and other medical research institutions.

Favorable working relations with Public Buildings Service representatives in both the Central and Regional Offices continue to be essential to the success of the NIH construction program. Therefore, constant effort is being made to improve and strengthen these relationships. The present General Services Administration arrangement of using Architect/Engineer personnel for the detailed inspection and supervision of construction under the general supervision of the PBS Region 3 Project Manager appears to be working satisfactorily. The new PBS Project Manager has rapidly become acclimated to the NIH program and has provided excellent support and counsel to date. General rapport and cooperation between the office of the Project Manager and the RFPB continue to improve.

The very detailed review and appraisal of proposed NIH construction plans by other agencies such as National Capital Planning Commission, Commission of Fine Arts, and Maryland-National Capital Park and Planning Commission, coupled with some differences of opinion between and within these groups, has significantly delayed progress on several projects during the past year. It is hoped that these can be resolved in the very near future.

II. BRANCH PROGRAMS

A. Objectives

The major objective of the RFPB is the coordination of planning and construction of new facilities on the NIH Reservation and at its field stations. This is supplemented with a secondary objective of providing development engineering services and technical assistance where special problems in

design or construction are encountered.

B. Current Programs

The design program is primarily responsible for determining that new construction provides for the program needs of NIH research scientists. A staff of professional engineers is employed to render limited consulting service and to provide coordination between contract architects, NIH research staff members for whom facilities are being provided, and other branches of the Division of Research Services.

The construction program provides close technical control of new facilities for NIH during the construction phases. A staff of management engineers maintains surveillance during construction to determine that the facility is built according to plans and specifications, with particular emphasis on those aspects peculiar to NIH requirements.

The current development program is directed toward improving the functional aspects of facilities presently under design as well as contributing standards, recommendations, and solutions to operational problems encountered in existing NIH facilities. Technical support rendered to the design and construction programs falls into a wide range of activities.

Members of the RFPB staff are frequently called upon to meet and confer with visiting research scientists and engineers relative to current concepts, techniques, and practices in the design and construction of biomedical research facilities. These visitors represent a broad spectrum of national and international medical research activities, both government and private.

C. Progress and Accomplishments (for a summary of all design and construction projects, see Table I and Table II, pp. 16-18.

General Office Building Extension (Building 31C)

This project, authorized in FY 1962 with an appropriation of $700,000 planning funds, was delayed at the Program of Requirements stage by the Bureau of the Budget, pending resolution of the vehicle parking requirements. However, in May 1963 the BOB approved the POR with "on-grade" parking, and the A/E design contract was negotiated in July 1963. Complete working drawings were transmitted in August 1965 and approved by the PBS in October 1965. At this point, it was decided to incorporate a portion of the required utilities construction with this project in order to insure a completed entity for occupancy and avoid conflicts during construction. Final working drawings of the building and the related master utilities were submitted in December 1965 and March 1966, respectively. Construction funds amounting to $5.5 million were provided in the FY 1965 appropriation. Construction is expected to start by August 1966. Beneficial occupancy should be effected in the spring of 1968.

Multilevel Parking Facility for Building 31

This project was authorized in FY 1966 with the appropriation of $111,000 for

planning a multilevel parking structure for 650 cars. The POR was prepared on the basis of an enlarged structure accommodating 1,150 cars; however, the BOB allowance for this facility, approved in December 1965, did not include the required increase in funds. Subsequently, the POR was revised to call for a capacity of 650 cars and in March 1966, with minor revisions requested by DHEW, was resubmitted through channels.

Multilevel Parking for NCI-NIMH/NINDB Buildings

Planning funds for this project, providing a multilevel parking structure for 825 cars, were appropriated in FY 1966. The POR was prepared on the basis of an increase in capacity to 900 cars; however, the BOB allowance for this facility did not include the required increase in funds. Subsequently, the POR was revised to call for a capacity of 825 cars and in March 1966, with minor revisions requested by DHEW, was resubmitted through channels.

Child Research Center (NIMH)

In March 1965, the Research Facilities Advisory Committee reviewed and approved the general layout and space arrangement as delineated in the diagrammatic sketches. The National Capital Planning Commission and the Maryland-National Capital Park and Planning Commission had originally approved the selected site. However, progress beyond the tentative sketches submitted on November 2, 1965, has been stopped pending the outcome of community-sponsored efforts to permit the extension of Woodmont Avenue through a portion of the NIH Reservation to Wisconsin Avenue. This extension would require resiting the CRC facility. NIH has agreed to the Woodmont Avenue extension and the A/E, Johannes & Murray & Associates of Silver Spring, Maryland, has been requested to study the feasibility of selecting a new site in the same vicinity, compatible with the research program.

Master Utilities Extension

This program is divided into two major phases: Phase I, which includes extension and expansion of utility systems required to supply those new facilities under construction, or recently completed, and Phase II, which was planned to provide utilities for the remainder of the buildings proposed under the Master Site Plan. The estimated cost for Phase I planning and construction is $7.5 million. One portion of the Phase I work was accelerated in order to meet the immediate needs of the Central Services Building Extension (Building 12A) and the Extension to the DBS (Building 29A). This work, Phase IA, was completed in January 1966 by Airmaster Services, Incorporated, Silver Spring, Maryland, at a base contract cost of $568,000.

To meet the scheduled needs of the construction program, other portions of Phase I have had to be placed on an accelerated basis. The final working drawings for the new chilled water plant, Building 34, and utilities for Buildings 35, 36, and 37 are completed. Construction will commence early in the summer of 1966. Final working drawings for the remainder of the work are expected in August 1966, with construction to begin in the fall. All Phase I construction should be completed in the fall of 1968.

Representatives of NIH and GSA have been negotiating a contract with the Washington Suburban Sanitary Commission for expansion of the water and sewer services to meet the increased demands of the NIH. These negotiations have extended over a period of two years and have required many discussions. Negotiations should be completed in FY 1967.

NIH Animal Center, Phase IA

The project includes a general laboratory building, animal behavior laboratory and waterfowl habitat for the NIMH, a primate building for Laboratory Aids Branch, DRS, and additional utilities and incinerator. After approval of revised tentative sketches in August 1965, this project was delayed because the A/E requested an additional fee and subsequently asked that his contract be terminated. Upon official notification in December 1965 to proceed with the design, the A/E resumed work. Intermediate working drawings were received by GSA and NIH in April 1966. Construction should start in the fall of 1966.

NIH Animal Center, Phase II

This project includes an agricultural management building, animal production facility, a waste chemical disposal facility, a third residence, and necessary supporting utilities and grounds improvements. The POR was approved by NIH and forwarded through channels to the BOB in November 1965. The BOB delayed approval of the POR and deferred action on a request for apportionment of the planning funds pending receipt of additional justification for the project.

Addition to Building 21

Work on the POR is in progress. After building planning was initiated, a decision was made to provide approximately 1,000 gross square feet for the Animal Hospital Section. Since the original budget request did not include funds for this additional space and the dollar ceiling for the whole project had been set at $500,000, some reduction in the space allocated for the Radiation Safety Department will result. Preparation of the POR is proceeding on this basis.

Warehouse Extension

The selected A/E for this design project is Palmer & Baker, Engineers, who were given the Notice to Proceed with the design on May 5, 1965. In the Diagrammatic Submission of June 1965, the A/E's cost estimate was $120,000 higher than the $670,000 available for the construction contract award. The PBS estimate at this stage indicated that the design would produce a building within available funds.

Due to the failure of PBS to give the A/E a Notice to Proceed with the development of a Master Plan for the warehouse area until October 1965, tentative sketches were not received until December 1965. The A/E's tentative cost estimate was $1,099,536, or $429,000 above the funds available for the construction contract award. Numerous methods have been explored by RFPB, PBS, and the A/E in an effort to reduce the cost of the project. All work on the project was suspended in January 1966 pending determination as to siting

and whether the requirement for a tunnel connection can be deleted. The proposed incinerator facility has affected the siting further.

NINDB/NICHD Research Facility, Puerto Rico

In FY 1963, design and construction funds for a building located at the Puerto Rico Medical Center, housing the NINDB Laboratory of Perinatal Physiology, were authorized. Legal problems with the transfer of the necessary land to the United States Government delayed submission of the POR to the BOB until March 1964.

In June 1964, the BOB approved the POR providing 26,000 gross square feet at a total cost of $850,000. An A/E firm was selected for the design of this facility, but no agreement was reached on the estimated construction costs. In FY 1966, NIH requested, and the Congress authorized, an additional $1.67 million to expand the project to accommodate the NICHD research program. The POR was rewritten and resubmitted through channels to the BOB in September 1965. The BOB approved the POR providing a facility not to exceed 56,000 gross square feet at a total cost of $2.52 million. In February 1966, DHEW approved reprogramming of $200,000 from the total funds for development of an experimental animal compound facility at Sabana Seca, Puerto Rico. Negotiations with the selected A/E firm for design of this project (NICHD/NINDB Research Laboratory) were held in April 1966. Design work is scheduled for completion by August 1967, and beneficial occupancy is expected early in 1969.

NICHD Research Facility

This project was authorized in FY 1966 with the appropriation of $1,000,000 for planning. A POR has been written and is currently being reviewed at NIH. The POR will be submitted for approval of PHS, DHEW, and BOB in July 1966. Construction funds will be required in FY 1968.

Biomedical and Behavioral Research Laboratories for NICHD at NNMC

This project was authorized in FY 1966 for inclusion in the FY 1966 operating budget of NICHD. The POR was completed and forwarded for approval in October 1965 and approved by the OD in January 1966. The Chesapeake Division, Bureau of Yards and Docks, U. S. Navy, was requested to act as Contracting Officer for design and construction. An A/E firm was selected and commenced design work in March 1966. Completion of design is expected by June 1966, and construction will begin shortly thereafter. Construction is expected to be complete by April 1967.

Biologics Standards, Building 29A

The construction contract for this facility, a three-story structure with basement, was awarded in March 1965 to the George Hyman Construction Company at a cost of $4,498,000. Construction of this facility is progressing satisfactorily, and by the end of the fiscal year was approximately 70 percent complete. It is expected that beneficial occupancy will be realized in December 1966.

Gerontology Building, Baltimore, Maryland

Located on the grounds of the Baltimore City Hospital adjacent to the main hospital building, this four-story structure with a two-story mechanical wing is being constructed by John McShain, Incorporated, at a contract cost of $6,989,000. The contract was awarded on October 31, 1965. Although hindered by inclement weather during its initial construction stages, progress to date has been satisfactory. The facility, presently 10 percent complete, is scheduled for completion in the fall of 1967.

NCI-NIMH/NINDB Buildings and Cafeteria (Bldgs. 35, 36, and 37)

The construction contract for this building complex was awarded August 27, 1965, to the Blake Construction Company at a total construction cost of $16,782,000, which includes the contractor's base bid of $14,728,000 and alternates valued at $2,054,000. The complex includes a six-story structure and basement for the NCI, a five-story structure and basement for NIMH/NINDB, and a cafeteria of one story with basement. The Notice to Proceed was issued to the contractor on September 18, 1965, and the scheduled completion date, November 16, 1967, allows 26 months for construction. Although construction was hindered during the winter months because of adverse weather conditions, the project is proceeding on schedule. Construction is approximately 15 percent complete.

Basic Research Laboratory, Lexington, Kentucky

Construction of this facility was completed in the fall of 1965.

Development Projects

Program studies, the majority of which are of short duration, are reported and distributed internally to interested NIH organizations and/or published in trade journals. Requests for available information from extramural activities are also honored. These generally originate through the Division of Research Facilities and Resources.

Limited research contracts continue to be an effective and efficient means for increasing the quality of accomplishment and conserving manpower and money. Such contracts also increase program flexibility and provide detailed specialization where required.

Collaborative Projects

Improving Animal Care Facilities (in collaboration with Laboratory Aids Branch).

The vital importance of laboratory animals to health research has accentuated the need for better quality animals. In recognition of this need, a continuous research and development program to improve animal care and facilities has been in progress. Program investigations are subdivided into categories as follows: (1) product and material testing; (2) prototype development; and (3) factors research. The present studies include: (a) the development of

caging systems for the NIH Animal Center, Phases IA and II; (b) the development of the automated equipment and care procedures with emphasis on watering, feeding, and cage cleaning techniques; and (c) testing and evaluation of standard commercially available products.

RFPB also provides technical assistance and advice to other NIH groups in preparing design guidelines, criteria, specifications, and standards for equipment and facilities.

As a means of stimulating new approaches and introducing new concepts, numerous visits to laboratory and animal facilities were made. Information from private institutions, as well as other government facilities, has served to foster novel ideas and solutions to operational, design, and construction problems here at the NIH.

Informational Reports

1. Fume Hood Exhaust Ducting
2. Specifications for Laboratory Casework Standardization
3. Animal Care and Equipment Catalogue*
4. Economic Analysis of Auxiliary Air Fume Hood Operation
5. Distilled Water Distribution Systems for General Research Laboratories
6. Laboratory Benchtop and Sink Materials Study
7. Wall Coating Survey
8. Floor Covering Survey
9. Research & Development Team Project Reports** (e.g. Primate Caging, Photoperiodism)

* In collaboration with SMB.
** In collaboration with LAB.

D. Problems

One of the most serious problems in the RFPB design program is the slippage which occurs in estimated design progress schedules. These extensions of the design postpone the construction project and beneficial occupancy of the facility. This, in turn, further delays the implementation of the research program for the client institute. As a result of these delays, greater construction costs are incurred due to the escalation.

RFPB has made an analysis of the reasons for these design delays. Two major factors appear to be the causes. The first is an unrealistic (optimistic) time schedule for review of the design. RFPB has encouraged the inclusion of more realistic review periods in the design contracts. The second factor is lack of personnel to properly review the design. Personnel in the Design Division of the Public Buildings Service, GSA, has been reduced in the past year by losses due to retirement and resignation. Vacancies created by these losses have not been filled. The PBS has also experienced an increase in its workload. The problem pertaining to the delays has been brought to the attention of responsible GSA officials regularly and continually by representatives of DRS and RFPB. No relief is in sight.

One additional factor in the delays can be attributed to the review of other groups such as the Commission of Fine Arts, National Capital Planning Commission, the Maryland-National Capital Park and Planning Commission and others.

Some examples of major delays are itemized as follows:

1. Insufficient funds to adequately design Phase IA of the NIH Animal Center contributed toward a delay from August 1965 to January 1966.

2. Considerable delay has occurred on the Warehouse project due to the indecision of NIH on the need for a tunnel connection as well as the site of the proposed incinerator facility.

3. The NIMH Child Research Center has experienced extensive delays due to disagreements between NIH and community groups (Maryland-National Capital Park and Planning Commission and National Capital Planning Commission) over the proposed siting.

4. Negotiations with the WSSC for additional water and sewer services, which are needed for the expanding construction program, have required constant effort and diligent follow-up to accomplish limited progress.

Continued action is being taken by the RFPB to alleviate these problems.

The Public Buildings Service, which has direct responsibility for construction contract administration, continues to encounter difficulty in hiring and retaining competent personnel for construction supervision and inspection. Current A/E contracts for design of NIH facilities include an optional provision for supervision of the construction by the A/E under the direction of the PBS.

E. Program Plans

Construction work will start in FY 1967 on a substantial number of projects which are currently in the design stage. Activity attendant to this increased construction program will require careful planning, coordination, and cooperation to avoid interruption and interference with NIH research activities. Additional staff members to meet the Branch responsibilities in this program have already been recruited and several others will be needed.

The current and future development program will continue to place strong emphasis on the design-support function for the NIH facilities planning, design, and construction program. Areas of investigation presently being studied in depth are as follows:

1. Specifications and Standards for Fume Hood Ducting
2. Ventilation Standards (ESB)*
3. Wall Coating Investigations (continuing study)

* Denotes collaborating organization.

4. Flooring Materials for Animal Areas and Other Applications (continuing study)
5. Planning Cage Washing Areas and Cage Washer Specifications (ESB)*
6. Caging for NIH Animal Center (LAB)*

* Denotes collaborating organization.

F. Publications and Patents

None to report.

TABLE I - RFPB - FY 1966 Program of Authorized Design Projects

Project	Design Completion Date	Construction Completion Date	Construction Cost*	Planning Cost	Total Project Cost	A/E Designer
NIMH/NINDB Bldg.	4/65	11/67	$ 8,620,000	$ 619,000	$ 9,239,000	SH & GA, Inc.**
NCI Building	4/65	11/67	8,867,000	610,000	9,477,000	SH & GA, Inc.**
NCI-NIMH/NINDB Cafeteria Building	4/65	11/67	836,500	53,500	890,000	SH & GA, Inc.**
NIH Animal Center Phase IA	5/66	9/67	1,325,000	125,000	1,450,000	Heery & Heery
NIH Animal Center Phase II	6/67	3/69	7,458,000	517,000	7,975,000	Not selected
Office Bldg. Ext. Bldg. 31C	1/66	4/68	5,850,000	350,000	6,200,000	K, L, & C and C, G, & Van A.**
NINDB-NICHD Res. Facility, P. R.	8/67	2/69	2,192,000	128,000	2,320,000	R, D, and C**
Sabana Seca Facility Puerto Rico	10/66	4/67	186,900	13,100	200,000	Not selected
NICHD NNMC, Ph. II	6/66	4/67	153,700	16,300	170,000	Loewer, Sargent & Assoc.
Gerontology Bldg.	5/65	9/67	8,142,000	358,000	8,500,000	C, S & D & LW, C & W**
Master Util. Ph. I	9/65	7/68	6,839,000	497,000	7,336,000	HDNA**
Warehouse Ext.	10/66	1/68	723,500	56,500	780,000	Palmer & Baker Engrs.
NIMH Child Res. Ctr.	10/66	5/68	930,000	90,000	1,020,000	J & M**
NICHD Res. Fac.	2/68	6/70	9,000,000	1,000,000	10,000,000	Not selected
Multilevel Parking Bldg. 31	3/67	7/68	1,489,000	111,000	1,600,000	K, L, & C and C, G, & Van A**
Multilevel Parking Bldgs. 36 and 37	3/67	7/68	1,985,000	125,000	2,110,000	SH & GA, Inc.**

+ See following page for footnotes.

TABLE I - RFPB - FY 1966 Program of Authorized Design Projects

* Includes construction contract and other construction costs.

** SH & GA, Inc. - Smith, Hinchman & Grylls Associates, Inc.
K, L, & C - Keyes, Lethbridge & Condon
C, G & Van A. - Carroll, Grisdale & Van Alen
C, S & D and LW, C & W - Cochran, Stephenson & Donkervoet and Lucius White, Clay & White
R, D & C - Isadore and Zachary Rosenfield, Fred S. Dubin Associates, and Efrain E. Perez-Chanis
J & M - Johannes & Murray & Associates
HDNA - H. D. Nottingham & Associates

TABLE II

NEW CONSTRUCTION - FY 1966

	Design Completion (Date)	Contract Award (Date)	Construction Schedule (Months)	Contract Completion (Date)	Contract Overrun (Months)	Contract* Amount	Contractor
Basic Research Lab.[1] Lexington, Ky.	3/64	8/19/64	11	7/14/65	2	$ 319,000	Barco, Inc.
Master Utilities Ext.[2] Phase IA	8/64	12/29/64	12	1/1/66	0	568,000	Airmaster Services, Inc.
Biologics Standards[3] Bldg. 29A	11/64	3/16/65	20	11/6/66	-	4,498,000	George Hyman Constr. Co.
NCI-NIMH/NINDB/Cafeteria[3] Bldgs. 35, 36, 37	4/65	9/18/65	26	11/16/67	-	16,782,000	Blake Constr. Co.
Gerontology Building[4] Baltimore, Md.	5/65	10/31/65	23	9/20/67	-	6,989,000	J. McShain Inc.
Biomedical & Behavioral[5] Research Laboratories NICHD-NNMC	5/66	6/30/66 (est)	9 (est)	4/7/67 (est)	-	132,000 (est)	--------

Architect/Engineer

1/ Lawrence D. Cammack
2/ H. D. Nottingham & Associates
3/ SH & G Associates, Inc.
4/ Cochran, Stephenson & Donkervoet and Lucius White, Clay and White
5/ Loever, Sargent & Associates, Inc.

* Does not include contingencies, supervision or planning costs.

DIVISION OF RESEARCH SERVICES

Summary of Branch Activities — July 1, 1965, through June 30, 1966

PLANT ENGINEERING BRANCH — R. R. Holliday, Branch Chief

I. SUMMARY

During FY 1966 the Plant Engineering Branch stayed abreast of all service demands without increasing numbers of personnel.

The most significant development was the increase in workload for engineering services. Construction performed by private contractors increased about fourfold. This additional load, represented by major improvements and new building construction, placed a heavy burden on the Branch engineering and planning staff.

II. BRANCH PROGRAMS

A. Objectives

The mission of the Plant Engineering Branch is to provide responsive support to the medical research mission of the National Institutes of Health by fulfilling, as expeditiously and as economically as possible, the functions described below.

B. Current Programs

Operation and Maintenance: This function consists of operating and maintaining the NIH physical, mechanical, electrical, architectural, and landscaping facilities.

Engineering: This function consists of developing and preparing plans and specifications for repairs, alterations, and improvements to NIH facilities, administering and inspecting work performed by private contractors, and providing engineering services as required.

Construction: This function consists of providing craft services (station labor) for altering, repairing, and improving NIH facilities.

C. Program Progress and Accomplishments

Construction By Station Shops: Requests for PEB services are received in the form of written work requests and by telephone. Telephone requests for minor services such as clogged plumbing, light bulb replacement, air conditioning malfunctions, and utility leaks are received by three decentralized area maintenance units. All written requests are received by one central work reception office.

An average of 1500 telephone requests are received each week, while written orders average about 360 per week. Almost all telephone requests and about

60 percent of the written orders require little or no formal planning (plans, special material, or detailed estimates of labor). About 1 percent of the written orders, however, require the services of professional engineers for planning, and most of these projects are performed by private contractors rather than by station shops. No additional personnel were employed during the year for station shop activities. An examination of material expenditures for these activities indicates that gross work output remains high. An apparent trend has developed which reflects a decrease in second quarter issues each year. Chart No. I reports material expenditure trends by quarter since 1961.

A good measure of the adequacy or responsiveness of the station shops service is the work in backlog. Experience has shown that an eight-week backlog will allow the most responsive service without sacrificing efficiency. Charts No. II and No. III report the backlog trend for the central craft shops since January 1964. Occupancy of Building 12A beginning in September 1965, and various other factors caused an increase in workload during December, January, and February; otherwise the backlog remained relatively constant, indicating that responsive service was provided.

Construction By Private Contractors: Most large jobs, estimated to require 300 or more manhours, are constructed by private contractors.

Work awarded since FY 1963 has been as follows:

Fiscal Year	No. of Contracts	Dollar Value
1963	271	1,500,000
1964	218	3,016,000 1/
1965	178	2,250,000
1966	130 2/	8,200,000 2/

1/ included new building construction, Bldg. 12A ($1,440,661)
2/ these figures include work awarded through March and estimates of work scheduled to be awarded prior to July 1, 1966, including the NCI Virus Leukemia Building ($3,175,000) and Library and Cafeteria Additions to Building 10 ($2,100,000).

Library and Cafeteria Extension, Clinical Center--In 1961, $335,000 was appropriated for an extension to the Clinical Center Cafeteria facilities. Design on the project was delayed in order to more fully evaluate the effect of changes in the management of patient and employee food facilities. In 1964, an additional $360,000 was appropriated to allow for separation of the two operations. Also in 1964, $900,000 was appropriated for planning and construction of new library facilities, and in 1966, an additional $650,000 was appropriated to allow expansion of the basement and sub-basement areas to absorb certain administrative type functions being displaced by creation of additional patient care area.

Since both Library and Cafeteria Extension were planned as adjacent additions to the south side of the Clinical Center, and since much of the utility work involved was common to both, it was feasible to combine them as a single

NATIONAL INSTITUTES OF HEALTH
Division of Research Services - Plant Engineering Branch

MATERIAL SUMMARY

Quarters (fiscal year)

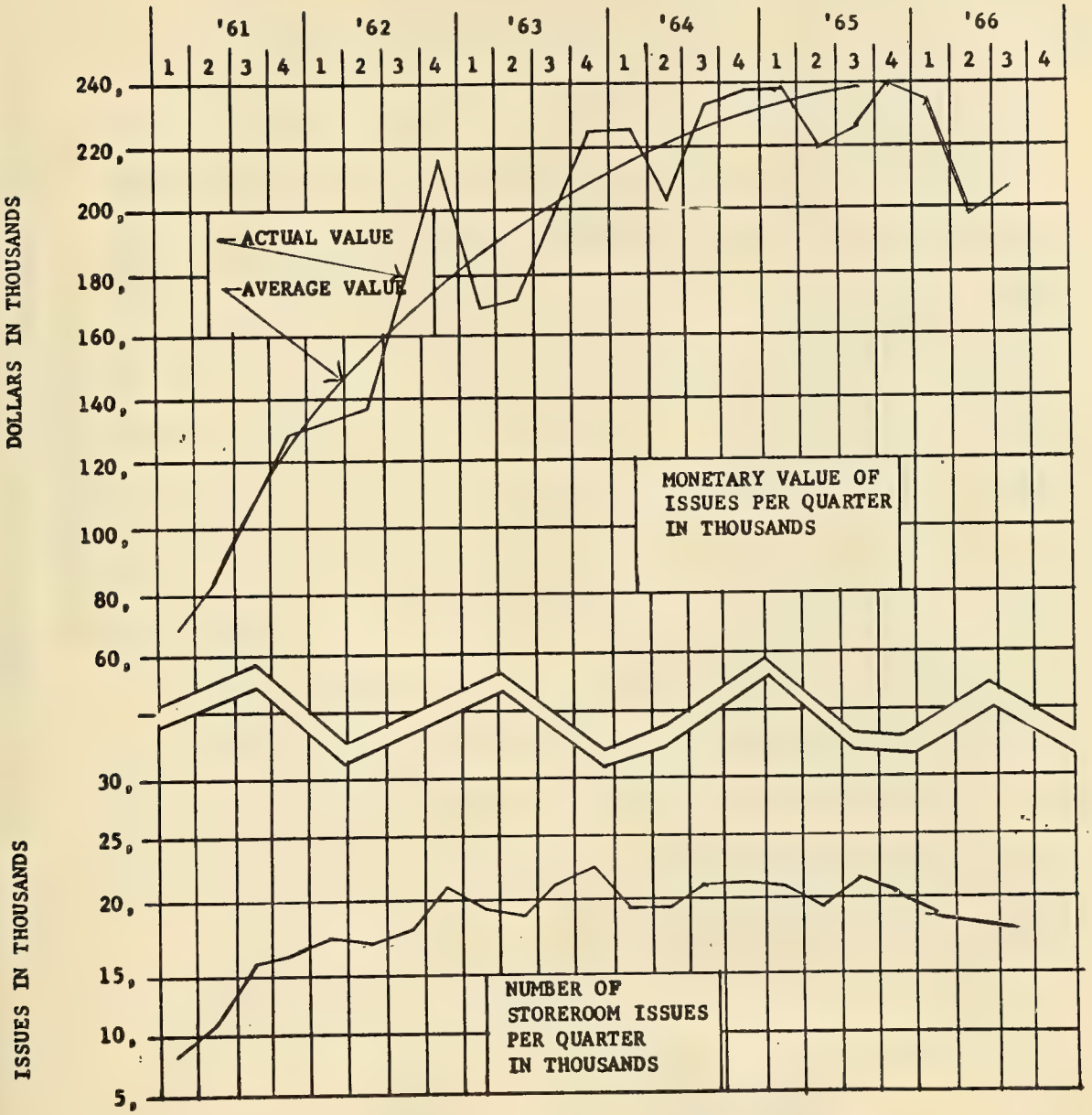


Chart No. I

NATIONAL INSTITUTES OF HEALTH
DIVISION OF RESEARCH SERVICES - PLANT ENGINEERING BRANCH

SHOP WORK BACKLOG SUMMARY

Reporting Period January 1964 thru February 1965

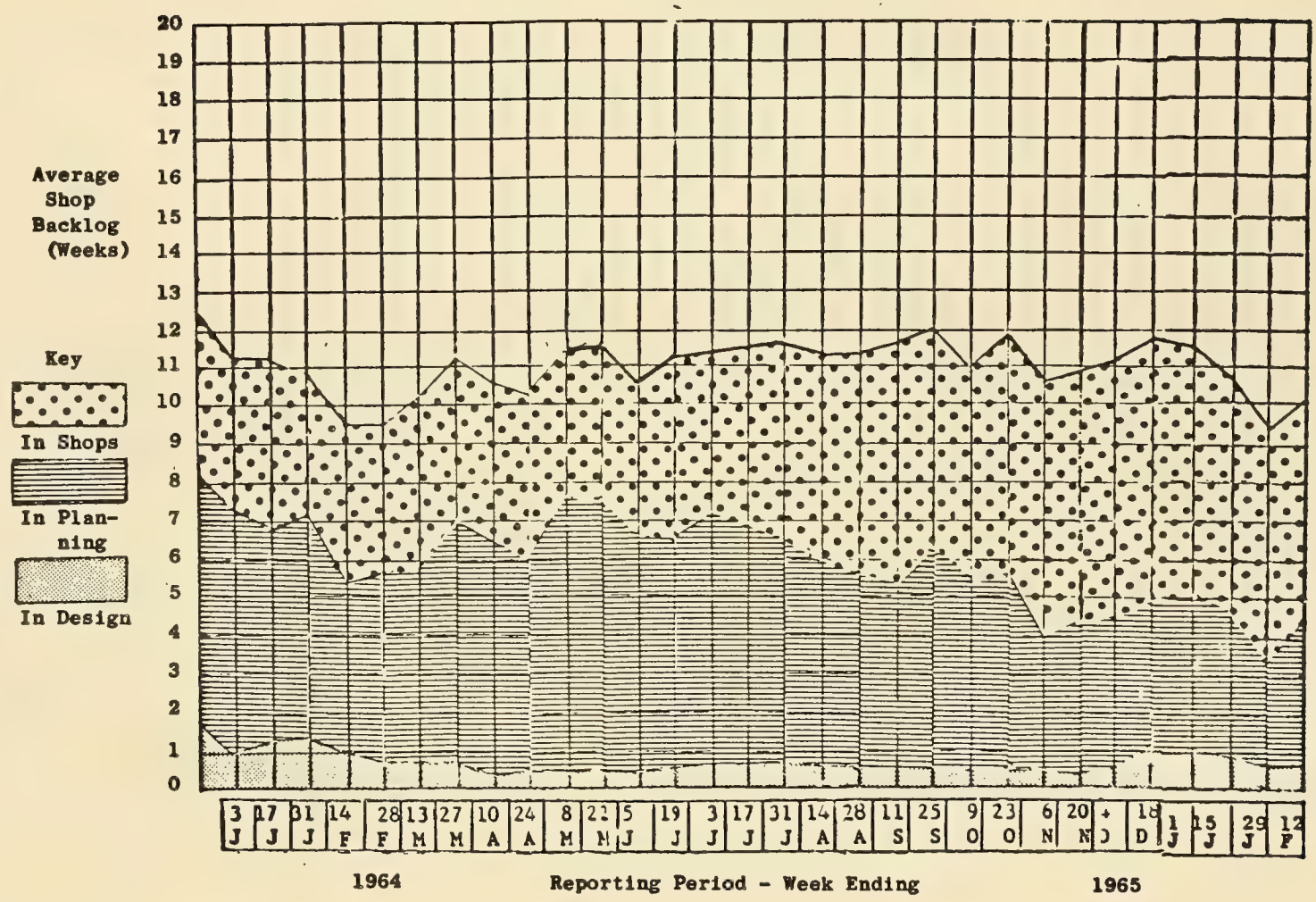


Chart No. II

NATIONAL INSTITUTES OF HEALTH
DIVISION OF RESEARCH SERVICES - PLANT ENGINEERING BRANCH

SHOP WORK BACKLOG SUMMARY

Reporting Period February 1965 thru March 1966

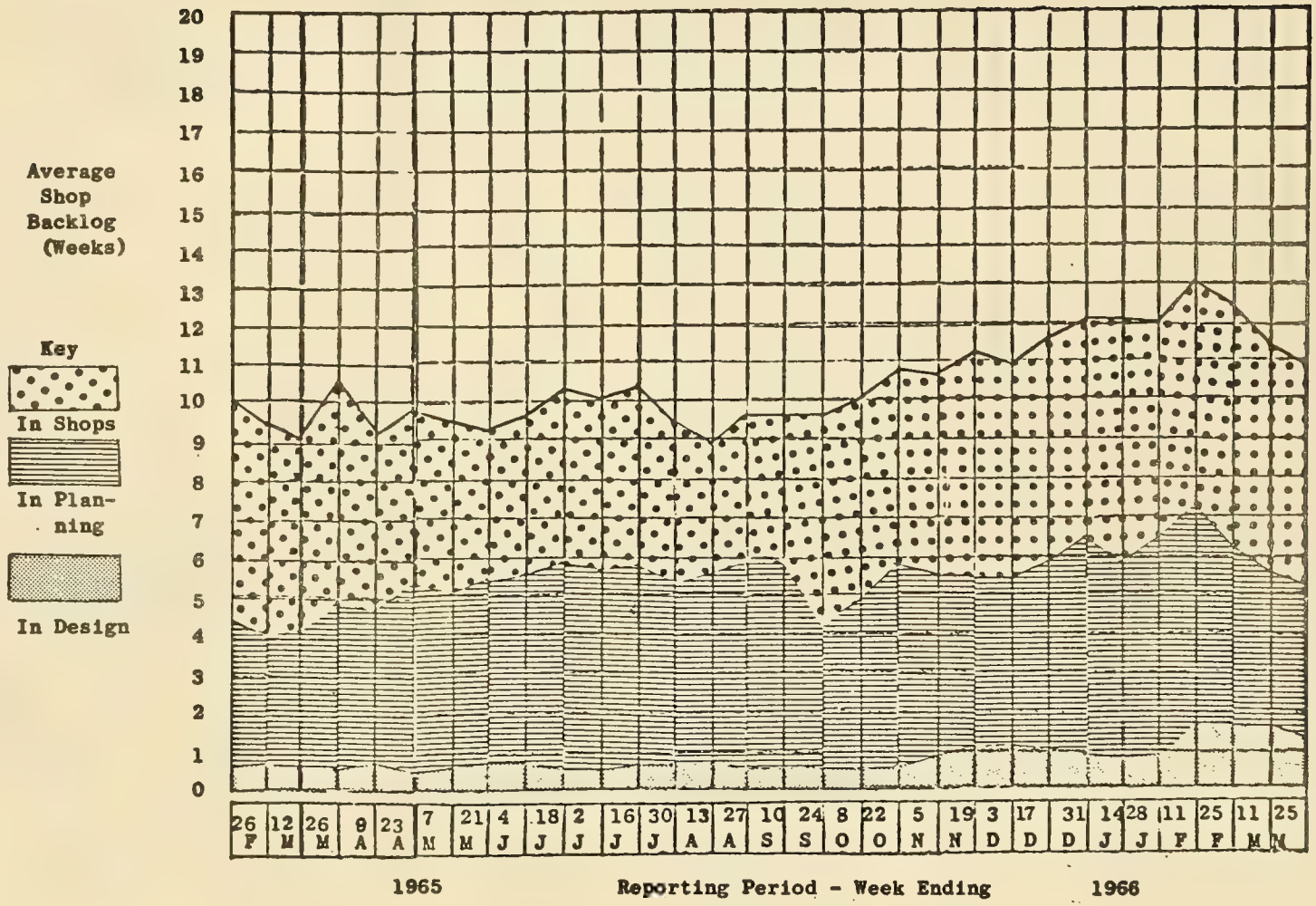


Chart No. III

project having total funds available in the amount of $2,245,000. The A/E contract for design was negotiated in July 1964, design was complete in November 1965, and a construction contract was awarded in April 1966. Construction is scheduled for completion in August 1967.

Wing D Addition, Clinical Center--In 1966, $900,000 was appropriated to expand Clinical Center facilities by adding two floors to the existing Wing D. This project involves removal of the existing sun deck and addition of the two new floors on top of the five currently present. Although this project was program-connected, to an extent, with the basement expansion of the Library, it was deemed more feasible to treat it separately because of the physical dislocation and the variance in the timing of the two.

The POR for this project was completed in November 1965, and an A/E contract for design will be negotiated in June 1966. It is estimated that construction will be complete by May 1968.

NCI Virus Isolation Facility--In 1965, a special appropriation of $10,000,000 was made to the National Cancer Institute to provide for design, mock-up, and testing of virus containment facilities and for construction of an emergency facility on the NIH grounds. Approval was requested for temporary construction authority for this project, and the approval was granted in April 1965. A request for assignment of responsibility for direct control of design and construction was made, and this too was approved in April 1965. Due to the proximity of the end of the fiscal year, no funds for design or construction could be obligated in 1965. By Congressional action, the funds were re-appropriated in 1966.

The POR for this project was completed in September 1965, and an A/E contract for design was negotiated in November 1965. Design will be complete in May 1966, and construction completion is scheduled for early 1968. The estimated cost of construction is $3,175,000.

Alterations For Direct Research Support: An assessment of the trend of this service can be made by comparing, for the past three years, annual expenditures for station labor materials and contract work.

Station Labor Material

FY	Material Expenditures
1963	$437,790
1964	486,180
1965	431,806
1966	515,000 *

Contract Expenditures (Awards)

FY	No. of Contracts	Costs
1963	212	$1,372,325
1964	126	659,458
1965	87	330,794
1966	57	367,677 *

* (projected by extrapolation for 12 months. Actual expenditure for first nine months - Station Labor $386,682 - Contract $190,000.)

From the above data it is evident that the level of services provided by station labor and by contract for alterations in direct support of research will be about the same as in 1965.

Engineering Design Services: During the report period, the professional engineering staff of the Branch prepared plans and specifications for contracted work, plus plans for larger station labor projects. This staff also directed the efforts of professional services contractors engaged by the Branch, and reviewed the work of professional services contractors engaged by others for the design of NIH facilities. A substantial amount of time was spent on this latter category due to the extensive building and utilities program underway.

Horizontal mobility of non-supervisory engineering personnel has been developed by revision of job descriptions which permit an architect or engineer to be transferred temporarily to any Section of the Branch requiring his skills. A result of this mobility is indicated by a 50 percent increase during calendar year 1965 in the man-hours of Engineering Design Section efforts chargeable to "construction time" to give assistance to the Construction Section with large construction projects.

A summary of the work for which design was completed during the report period follows (projected thru June 1966):

Type	Quantity Completed	Actual or Estimated Cost of Construction
Professional Services Contracts	11	$6,232,000.
Staff Design Projects for Contract Work	93	2,158,000.
Staff Design Projects for Shop Work	91	48,000. (Materials only)
Engineering Studies or Surveys	19	
		$8,438,000.

A few of the more important projects in each category are listed below:

Alterations	Actual or Estimated Cost
Construct RIF-Free Chicken Houses, NIHAC	$80,000.
Social Observation Building, NIHAC	75,000.
Locker and Washroom Facilities, Building 1	21,000.
Install 360/30 IBM Computer, Building 12	30,000.
Renovate Computer Space, Building 12	22,000.
Alter Labs on 6th and 7th Floors, Building 10	70,000.
Relocate Viral Oncology Facilities to NIHAC	100,000.
Renovate Dental Clinic, Building 10	50,000.
Plant Maintenance	
Paint Public Areas, Buildings 1, 2, 5, 8, 11, 13, 31	21,000.
Repair Work on Boilers, Building 11	30,000.

Plant Maintenance (continued)	Actual or Estimated Cost
Modernize Motor Control Boards, Buildings 3, 4, 5	$15,000.
Alterations to Elevators, Building 10	40,000.
Roof & Gutter Repairs, Buildings 1-8	30,000.
Correct Exterior Wall Condensation, Building 10A	10,000.
Alter Can Handling Facilities, Building 11A	75,000.
Install Rotoclone System on Incinerator Waste Stack	70,000.

Improvements and New Construction

Library Addition and Cafeteria Extension, Building 10	$2,100,000.
Modify Pneumatic Tube System, Building 10	170,000.
Emergency Virus Isolation Facility	3,175,000.
Fire & Safety Improvements, Bldg. 10 (Phase II)	100,000.
Modify Boilers and Cold Storage Rooms, RML	190,000.
Improve Passenger Elevators, Building 1	225,000.
Convert Building 6 to Central Plant Chilled Water	90,000.
Replace Galvanized Waste Stacks, Building 10	225,000.
Renovate Electrical Distribution System, RML	35,000.

Engineering surveys were conducted to resolve problems of vibration or electrical interference, to assist operational personnel in determining causes of malfunction of equipment, and similar problems. Also, many investigations were made to determine the feasibility, alternatives, and costs of proposed projects.

Procurement Activity: During the first three-quarters of the year, the Shop Stores Unit, PEB, purchased directly 93% of all line items purchased involving 33% of the dollar value. The remaining procurement was made by the Supply Management Branch. Procurement for the first nine months of this year totalled approximately 7000 line items costing $550,000. Total procurement for the year is expected to exceed $1,000,000. This level of activity was about the same as 1965.

Employee-Management Relations: The first agreement involving NIH under Executive Order 10988 was negotiated by the Branch with the Washington Area Metal Trades Council, AFL-CIO. The agreement covers all eligible non-supervisory wage board employees of the Grounds Maintenance & Landscaping Section, PEB.

D. Problems

PEB Material Pre-Packaging Program: During FY 1965, preliminary steps were taken to initiate a material pre-packaging program. Purpose of the program is to reduce the lost time resulting from shop employees having to wait in line at the Shop Stores issue counter when drawing out repair parts and construction materials. The program is set up to operate in the following manner:

1. For the jobs that lend themselves to the procedure, the planner-estimators will plan the work in much greater detail than before and will call out all standard stock material as well as special material.

2. Upon receipt of the orders, the shop planners will designate which items are to be packaged together and pre-issued. Normally the breakdown will be by craft specialization (plumbing, electrical, sheet metal, etc.). In some cases where a particular trade must stop work because of interference with other trades, the material for that trade will be pre-issued in more than one package.

3. The storekeepers will draw the material, package it as indicated by the shop planners, and move it to the special storage area to be held in custody storage. The storekeepers should be able to do this work when there is a lull at the regular issue counter.

4. When production work on the job is scheduled to begin, the craftsmen for each trade will draw out the appropriate package of pre-issued material with a minimum of waiting time.

During FY 1966, an implementation schedule was developed, the basic criteria were established, and several pilot jobs were processed under the procedures. Initially, the program is being limited to pre-selected shop jobs involving over 250 man-hours of station labor. It has been found that additional planning time, averaging about four man-hours per trade specialization (architectural, plumbing, mechanical, electrical, etc.), is required. Within the availability of storage space and other limitations, this program is scheduled to be in full operation by July 1, 1966.

PEB Tool Control Program: In FY 1965, major planning for a formal tool and equipment control program was completed. The toolroom mechanic who will operate the central PEB Toolroom entered on official duty on January 3, 1966. The tool crib area was cleared, and a substantial start was made in inventorying the tools and equipment to be included in the program. Initial requirements for storage cabinets and shelving were determined, and the cabinets and shelves were ordered. A schedule for implementation of the program was developed. Charge-out procedures on a limited basis for the items on inventory were begun on February 11, 1966. Full operation of the system PEB-wide, including the operation of several satellite toolrooms, is scheduled to be in effect July 1, 1967.

Pre-Expended Material Program: The original PEB Shop Stores system made provision for pre-expended, small-value, and non-pilferable materials located in several areas throughout the Branch. The purpose was to reduce waiting time of journeymen at the storeroom issue counter. This activity had been handled informally, and a review indicated the procedures were not very effective.

During FY 1966, steps were taken to modify the procedures so as to make available more useful and popular items located in more suitable locations. The Shop Stores Unit will exercise more management effort to make this program more effective. An implementation schedule was developed to keep the program under continuous attention. Lists of new items to be included were prepared for four stations in Building 13. New storage fixtures (shelving and bins) were ordered, and these stations are scheduled to be in full operation before the end of the fiscal year.

During FY 1967, it is planned to extend the program to such other areas as the Power Plant Unit, the Building Units, and Grounds Maintenance and Landscaping Section, with full implementation scheduled by June 30, 1967.

Uniforms for PEB Wage Board Employees: Over a period of years, the practice had developed whereby work uniforms and laundry service were furnished at government expense to a limited number of PEB employees, mostly in the Grounds Maintenance and Landscaping Section and Clinical Center Unit, Maintenance Engineering Section. During the year, the Washington Area Metal Trades Council, which has been granted formal recognition for PEB wage board employees (except in Grounds Maintenance & Landscaping Section where they have been granted exclusive recognition), requested that all wage board employees be provided with uniforms and laundry service.

Basic authority to provide uniforms and other apparel is covered by Section 509 of the Public Health Service Act which reads, in part, as follows:

> "Section 509. Appropriations for carrying out the purposes of this act shall be available for .....furnishing, repairing, and cleaning such wearing apparel as may be prescribed by the Surgeon General for use by employees in the performance of their official duties ....."

The policy was adopted that all wage board employees would be required to wear suitable clean apparel which will (a) identify the persons as being employees of NIH and PEB and, (b) insure that the employees, who may be subject to contact in varying degrees with infectious or soiled materials, will have an adequate supply of clean clothing available when required. To enforce this requirement on a fair and equitable basis, the uniforms will be furnished and cleaned at government expense.

The initial supply of new uniforms was ordered for issue at the beginning of the warm weather season. They are dark green in color, and many items of clothing are identical to those previously furnished to the Grounds Maintenance personnel.

Recruitment: In July 1965, the Branch had a total of 36 vacancies, and in April 1966, a total of 34 vacancies. During this period, 52 new employees were hired while 50 employees were lost through transfer, retirement, death, etc.

In order to cope with the major NIH expansion (32%) projected for FY 1968, which tentatively is programmed to require 134 additional PEB personnel, it will be necessary to improve both retention and recruitment of personnel. Retention of personnel is greatly affected by the practices of local government agencies in competing for available skilled craftsmen and by the overall supply of skilled craftsmen. The DRS Personnel Officer is aware of certain practices which tend to damage our competitive position, and has already taken action in one area. Additional steps may become necessary.

Retention alone, however, will not result in attracting the large numbers of additional craftsmen which will be needed to provide services for a greatly

expanded National Institutes of Health. It is our intention, therefore, to push vigorously for establishment of an apprenticeship program with the twin goals of expanding the range within which we may recruit and improving the competence and capability of our craft workers.

Training: The Branch supported approximately 567 man-days of formal classroom training for FY 1966. In-service training amounted to 449 man-days, consisting of attendance at the Management Grid Course, Secretarial Workshop course, supervisory courses, and various clerical courses on the Autotutor machine, as well as 270 man-days which were provided within the Branch itself in a thorough course in Refrigeration and Air Conditioning. The remaining 118 man-days consisted of non-government training, with engineers taking a high percentage of these courses.

This type of training has proved its worth and will be continued. However, additional training is still needed both in the "pre-journeyman" stage and in the supervisory areas. It is planned to alleviate the first of these needs by an apprenticeship training program aimed at developing competent workers in each of the trades and crafts. Supervisory training will be provided through the local presentation (by a member of the Plant Engineering Branch) of a course developed by the Civil Service Commission, entitled "Supervision and Group Performance." This is a 40-hour course designed for a class of 16 to 20 participants. The first presentation was scheduled for the week of June 6, 1966, with additional presentations to follow until all PEB supervisors below the level of Section Chief have had the training.

Incinerator Plant Capacity: A study made by the Branch during the year resulted in an estimate that the capacity of the existing plant will be exceeded during 1968-70. The load by 1968-70 is estimated to be about 1000 tons per month. The maximum capacity of the plant is about 850 tons per month. Funds have been included in the FY 1967 budget for planning a new facility. It is estimated, however, that the plant will be overloaded before a new plant is built. If this occurs it will be necessary to segregate refuse and contract with a local municipal plant for disposal.

Steam Generating Plant Capacity: Studies during the year resulted in an estimate that the plant capacity of 300,000 pounds of steam per hour will be exceeded during calendar year 1968 with the additional loads imposed by Buildings 29A, 31C, 35, 36, and 37. To meet this need, an additional boiler is to be installed as part of the overall utility updating (MUE). If the new boiler is not installed before the load exceeds present capacity, the standby boiler will have to be used. With this arrangement, a failure of any boiler would result in the need to cut down on the steam supplied to some areas during peak loads.

NIH Water Supply: Since 1961, the condition of the water supplied by WSSC has deteriorated as a result of the switch-over of the main source of supply from the Patuxent River to the Potomac River. This situation has overloaded water softeners, deionizers, water stills, and related equipment. Efforts are now being made to increase the capacity of this type of equipment, part of which is located in the various laboratory buildings, and the remainder of which is in the central utility plant, Building 11.

Grounds: The extension of the drought through the third year (1962-1965) has had an adverse affect on the condition of the grounds. In addition, the extensive trenching and excavation for new construction and shortage of personnel for maintenance have contributed to the problem. The following steps are being taken to alleviate the situation:

1. Additional personnel have been authorized and employed.

2. All lawn areas are being aerified to a depth of three inches, leaving a pulverized mulch which improves water penetration and reduces run-off during heavy rain.

3. Non-ionic organic wetting agents are being applied to lawn areas to promote water penetration and percolation.

4. Fertilizers and growth inhibitors are being selected to promote root growth and inhibit plant growth to reduce moisture needs and improve plant health.

5. Many trees are being transplanted, grades adjusted, and tree wells and barriers built to protect trees from excavation.

E. Program Plans

Operating Plans: Long- and short-range plans used to direct the day-to-day business of the Branch include: (a) operating budget and related reports; (b) daily reports from the preventive maintenance system as needed to generate the maintenance needs requiring immediate attention, and monthly and annual reports for major repair and improvement program plans; (c) weekly workload reports which reflect the estimated craft labor requirements by trade; (d) monthly workload reports which reflect the estimated engineering manpower requirements for engineering services by discipline; (e) weekly personnel status reports which report the available manpower by organizational unit resulting from personnel attrition and recruitment; and (f) monthly Shop Stores status reports. The Branch developed written step-by-step instructions for the purpose of standardizing the methods and sources of obtaining basic information required in the preparation of all material expenditure and manpower utilization reporting documents.

F. Publications and Patents

None.

Summary of Branch Activities July 1, 1965, through June 30, 1966

ENVIRONMENTAL SERVICES BRANCH Edwin M. Lamphere, Sr San E
Branch Chief

I. SUMMARY

The Branch continued to approach its objective primarily by solving, on a short-term basis, problems brought to it by researchers and others outside the Branch. Visible progress is generally more apparent at the conclusion of long-term projects. However, the researcher's present needs cannot be ignored until major changes are brought into effect. Work on long-term solutions was increased during FY 1966.

To increase management effectiveness, insect and rodent control operations were transferred to Office Services Branch, OAM. This move relieves the Environmental Services Branch of the responsibility of inspecting its own work. It also allows Branch management more time to concentrate on its professional-level activities.

Greatest program progress was made in activities associated with control of biological hazards. In addition to numerous short-term solutions, Building 7 was evaluated with regard to its suitability for hazardous work, and a suite for hazardous work was developed for Building 6.

Quality of the potable water supplied to NIH has resulted in many problems over the past years. In order to arrive at a concerted course of action, a study of the problem on a reservation-wide basis was completed.

The laminar air flow concept developed for industrial clean rooms appears to offer a method for improving control of biological air contamination at NIH. A mock-up operating room using the laminar flow type of ventilation system was constructed so that new ideas may be evaluated.

Air pollution generated by the NIH power plant has been a nuisance problem in the past, and with completion of new construction it would have become a real problem in the technical sense. However, an investigation which defined the scope of the problem was completed and is serving as the basis for decisions on required changes.

With research requiring higher and higher quality animals, factors associated with contamination in production colonies become increasingly important. The Building 14 production areas were surveyed to provide methods for solving potential problems in existing facilities and to provide information for use in the design of new facilities.

II. BRANCH PROGRAMS

A. Objective

The objective of the Environmental Services Branch is to insure that a sanitary and otherwise satisfactory environment is maintained at NIH and its field stations.

B. Current Programs

Attainment of the Branch objective was approached through six closely coordinated functional program areas:

1. Occupational health engineering adapted specifically to the NIH clinical and laboratory research needs

2. Hospital sanitation

3. General sanitation

4. Biological hazard control

5. Water supply--both potable and special purpose

6. Waste disposal--both liquid and solid

Administratively, the Branch approached its objectives by serving as consultant to line management in the Institutes and Divisions. Recognizing that Institute and Division management has a wide variety of problems other than those related to the environment, it is the policy of the Branch to furnish, in addition to a statement of the problems, practical solutions tailored to the individual administrative and technical situation. For problems involving more than one Institute, this approach often leads to development of different solutions for the same problem. However, the results warrant the extra effort.

Most of the Branch workload continued to be of a trouble-shooting nature, whereby an individual problem is investigated and solved at someone's request. However, more time is being spent each year on surveillance activities and on solving problems on a larger-scale basis, which ranges from one wing of a single building to reservation wide.

With regard to the technical approach, occupational health engineering is primarily involved with problems involving air pollution, toxic and noxious fumes, toxic particulates, odors, ultraviolet lighting, illumination, and ventilation.

Hospital sanitation activities are concerned only with the patient-care areas of Building 10, and deal with the infection control aspects of the environment. Routine quarterly surveys are conducted in all operating rooms. Routine monthly surveys are carried out on six of the nursing units where the potential for infection problems are highest.

Approximately half the time devoted to hospital sanitation is spent on various surveillance activities. The other half is spent in dealing with problems of an emergency nature and in developing corrective measures for potential problems.

General sanitation activities are carried out throughout the NIH reservation, NIHAC, and other NIH facilities in nearby areas. During the first part of this year the work followed the past pattern of emphasis on the conventional sanitation aspects of infectious waste disposal, animal rooms, cafeterias, snack bars, vending machines, and housekeeping practices. During the latter part of the year a shift was made toward integrating the general sanitation work with Branch responsibilities in the control of biological hazards. The same areas are covered, however.

NIH central staff aspects of controlling biological hazards were added to the Branch responsibilities in August. This work consists of general surveillance of potentially hazardous areas, special building surveys, development of facilities to provide safe conditions, and general problem solving on request.

Water supply activities are concerned primarily with problems involving the quality of potable and distilled water. In an institution as large as NIH, and with the high quality of water demanded by research work, there is always some type of water problem.

Waste disposal activities involve solid wastes generated by the research laboratories and animal rooms, the NIH sewer system, and the sewage treatment and disposal system at the Animal Center.

C. Program Progress and Accomplishments

Program emphasis within the Branch continually shifts to reflect the changing requirements of the NIH research program and different stages of development of on-going Branch responsibility areas.

Because of the increasing amount of research work involving potentially hazardous microorganisms and the new Branch responsibilities in this area, activities associated with control of biological hazards were given a high priority; and other activities were de-emphasized accordingly. The only 1965 program area to escape the de-emphasis was hospital sanitation, which, as the program is operated, is a biological hazard control effort aimed specifically at the NIH clinical research functions.

Insect and rodent control operations were de-emphasized to the point of requesting that this responsibility be transferred to Office Services Branch, OAM. The Branch program has evolved into what is essentially a professional-level surveillance and problem-solving operation. Good management practice demands that surveillance and the operations which are subject to surveillance should not be located within the same organization. Management efficiency was increased by the transfer, since there is no longer a need for the dual role of managing a professional group with one set of problems and a nonprofessional group with an entirely different set of problems.

Of the remaining Branch programs, lowest priority was given to water supply and to general sanitation. Water supply was selected for a low priority because the NIH problems in this field are rarely of importance to the health of employees or hospital patients. General sanitation received a low priority because the same types of personnel are needed for both general sanitation and biohazards control. While some problems developed during the year as a result of de-emphasizing general sanitation, in the long-range context it will prove to be an advantage. Sufficient time was spent on this activity to avoid any serious problems. In order to attain maximum efficiency, this activity would have had to be eventually reoriented to dovetail with the biohazards activities. This probably can be accomplished much faster in a rebuilding process than by attempting to change the direction of a vigorous on-going program.

While the Branch workload continues to consist mostly of investigating and solving relatively small problems, more and more time each year is spent on long-range solutions. Handling problems on an individual basis will always result in a large workload, since NIH research work can't wait until major changes are brought into effect. However, on an overall basis, short-term solutions result in staying a little behind the problem rather than ahead of it. Visible progress in upgrading the environment generally depends on longer-term efforts.

Long-range solutions include, but are not restricted to, the strictly technical aspects of NIH's environmental problems. Training of laboratory technicians and animal caretakers provides long-range benefits which cannot be achieved in any other way.

Routine surveillance uncovers potential problems before they become actual problems. Special, relatively large-scale surveys allow problems to be solved on an economical large-scale basis. Development of equipment such as special hoods also provides long-range solutions. Industry has proven to be cooperative on this type of development, but industry does not have the close contact with the NIH scientist which is needed to initiate such work.

Routine service-type functions have gradually been transferred to other NIH groups over the years to allow increased emphasis on other aspects of the Branch program. Of the original service functions, little remains but distilled water surveillance. However, this type of work started to increase again in 1966 as a result of biohazards control work. An important aspect of controlling biological hazards is the decontamination of potentially hazardous spaces and equipment. While the vast majority of decontamination is carried out by the individual laboratory, an institution as large as NIH provides enough special work to require that manpower be assigned to it. Provisions were made during the year for (a) converting the large Branch autoclave to ethylene oxide, so that materials which could be hazardous to patients do not have to be taken to the Building 10 autoclave; (b) development of a sealed plastic container for enclosing potentially hazardous equipment prior to decontamination; and (c) collecting a complete kit of materials to be used in either emergency or routine decontamination of laboratories and animal rooms.

The success of any of the environmental control programs is dependent on acceptance by the Institutes. Acceptance of the biohazards program has been excellent. While the Branch cannot take complete credit for this, it can take credit for developing good relations with the Institutes.

At the request of NIAID, a complete evaluation of the suitability of Building 7 for potentially hazardous work was made. ESB has worked on a daily basis with the NCI personnel assigned to the development of the new virus containment building. A complete suite in Building 6, including the development of special animal cages, was worked out to allow virus research activities to continue until the new virus containment building is completed.

Although at the time the new Surgical Wing was completed, its operating rooms were the best in existence with regard to control of air contamination (and still may be), further progress appears possible by use of the laminar air flow concept. The Branch has constructed a mock-up operating room using laminar air flow, and a cooperative project is underway with NINDB to compare the results of the two different types of air supply systems. While the ventilating system in the new Surgical Wing is very good, a quirk in the design allows the system to malfunction on an intermittent basis. The source and results of the malfunctions were determined and remedial action was initiated.

A potential infection problem in the Clinical Center was traced to several patients who were shedding organisms in large quantities. This pinpointing of the problem allowed the necessary control action to be taken.

Air pollution at NIH has been a nuisance and, therefore, somewhat of a problem for several years. However, the steam requirements for new NIH construction have changed the situation into an actual problem for the immediate future. Arrangements were made with the Division of Air Pollution, PHS, for a meteorological study, and this has provided the information on which to base decisions for required changes.

Water supplied to NIH by the Washington Suburban Sanitary Commission has resulted in a large number of problems because of the intermittent high levels of suspended solids. In the past, these problems were treated individually. This year a reservation-wide study of the problem, including economic considerations, was completed. Based on estimates of the problem received from the individual Institutes, it was concluded that re-filtration of all water could not be justified and that greatest possible centralization of water-using equipment would be the most appropriate solution. In this way, adequate filtration could be provided at a cost which is somewhat commensurate with the problem.

To provide information on which to base a contract with the Washington Suburban Sanitary Commission, sewage flows at NIH were measured, and methods were developed for estimating these flows at any stage of NIH's development. While methods for estimating sewage flows from a municipality are well established, these are not even remotely applicable to NIH.

Even excluding germ-free and pathogen-free animals, efforts must be made to provide the researcher with an ever-improving quality of animal. Under these circumstances, environmental considerations become particularly important. A detailed survey of the animal production areas in Building 14 was completed. This provided information necessary to avoid potential problems in existing facilities as well as concepts to be incorporated into the design of future facilities.

D. Problems

Most of the problems encountered during the year resulted from a shortage of trained personnel. Lack of sufficient personnel ceilings during the first part of the year to staff the new biohazards program and, at the same time, carry out essential functions of other programs contributed to the problem but was not the sole cause. Personnel trained in any of the phases of institutional environmental control are in very short supply, and generally it is necessary to recruit personnel who have only the basic background knowledge upon which competence can be built. On-the-job training must be carried out over a period of several months to a year before the new employee becomes fully productive at his grade level.

Aside from the army biological warfare operations, there is almost no other manpower pool from which to recruit personnel for biohazard control activities, and even these personnel would require a considerable amount of reorienting to be useful in the NIH program.

While the field of hospital sanitation is receiving more and more interest at the present time, the hospital sanitarian who is competent to go into a hospital operating room and evaluate the situation does not exist outside of NIH, for all practical purposes. Occupational health engineers who could quickly adapt to NIH needs exist, but government salaries do not compare favorably with those paid by industry.

Fortunately, recent engineering graduates of excellent quality are in plentiful supply through the Commissioned Corps recruiting channels. However, about a year's training is usually required before they become really competent in the health-oriented aspects of engineering needed by the Branch. The engineering career development plan calls for two year assignments.

Plans for improving the situation differ with the various categories, and expected success rates range from very optimistic for the immediate future to fairly optimistic for the distant future. Plans to alleviate the problems in the sanitation aspects of biohazards control consist of in-depth staffing. By combining the activities of the biohazards sanitarians and general sanitation, this can be accomplished. One or two trainees in a group of four or five can be handled easily. This plan is already proving effective.

With regard to engineers, plans are underway to develop an intra-divisional career development plan which will take advantage of training in other Divisions. This has been cleared in principle by the Office of the Chief, DRS.

It is probable that ESB will never be able to justify in-depth staffing in hospital sanitation solely on the basis of the size of that program. However, staff expansion sufficient to provide back-up support in this program may become necessary because of the problems with recruitment of competent hospital sanitation personnel. Because of the present limited staff size, each staff member is responsible for specific areas of the program, and loss of one staff member results in loss of that area of coverage until such time as a replacement can be recruited and trained. Development of hospital sanitation programs in other hospitals is being encouraged with the hope that a manpower pool can be created and used as a recruitment source. However, it will be years before such efforts bear fruit.

E. Program Plans

No change is foreseen in the objectives of the Branch. However, key programs which were de-emphasized during this year will be brought up to last year's level in terms of manpower and hopefully above that point in terms of effectiveness.

In order to staff the biohazards program, personnel were transferred from occupational health engineering and general sanitation. The vacancies created in these areas are now being filled, and, provided that recruiting is successful, water supply and liquid waste disposal will be the only low priority programs at the end of another six months. Highest priority will again be given to the health-related aspects of environmental control, but more work involving control of the environment as it affects the research project appears to be possible.

A small-scale reorganization within the Branch is contemplated during the fall of 1966. All hospital activities, both engineering and sanitation, will be placed in one unit which will be managed by an employee who will have received his Ph.D. in hospital engineering by that time. For a variety of reasons, it is difficult to coordinate the engineering and sanitation aspects of the hospital work when they are in different sections.

In addition, it is planned to consolidate biohazards surveillance activities and general sanitation activities. The two are similar enough that it would be wasteful of manpower to continue to keep them separate. The combination will provide a sufficiently large staff trained in biohazards work to cope with emergencies. The in-depth staffing will minimize the personnel training lag time.

Summary of Branch Activities July 1, 1965, through June 30, 1966

LIBRARY BRANCH

Jess A. Martin
Branch Chief

I. SUMMARY

The fiscal year covered by this report saw further implementation of the internship, bibliographical services, automation, and workload reporting programs. Equipment lists and specifications were prepared for the new library. Tentative plans were developed for moving personnel, collection, and equipment to the new library building without a major interruption of service. Heavy emphasis was placed on staff training programs during the year.

Changes in the Library's policies and methods of operation made possible a relaxation of some of the regulations that had been developed during the earlier reorganization period. All program changes during the year were again geared to the concept of providing quality service to the research staff of the National Institutes of Health.

II. BRANCH PROGRAMS

A. Objectives

The primary mission of the Library Branch is to operate an efficient, comprehensive library to support the research programs and administration of the NIH Institutes and Divisions. Its activities include the acquisition, processing, maintenance, and circulation of library materials; the operation of a photocopying service; the provision of information, reference, and bibliographical assistance; and the provision of a translating service for foreign scientific literature. To fulfill its mission, the Library must be responsive to the changing literature needs of the NIH investigator and knowledgeable of the current information retrieval, manipulation, and dissemination methods applicable to library procedures and services.

B. Current Programs

Readers Services. Readers services for the NIH staff include reference and bibliographical assistance, circulation and maintenance of literature, photocopying, and translations.

The reference staff compiles short bibliographies on specialized subjects and performs comprehensive literature searching as requested by NIH investigators. The comprehensive literature searching frequently involves the formulation of requests for Medlars by NIH librarians trained at NLM to formulate the requests. In order to answer the multitude of inquiries for specific information, the reference librarians endeavor to keep abreast

of the activities of NIH and other research institutions and of biomedical developments through attendance at lectures, personal reading, and course instruction. In this way they are also prepared to make contributions to the book and journal selections program. As much contact as possible is maintained with the scientist in order to appreciate his specific library needs.

The Circulation Unit, through surveillance and inventory of the collection and its use, maintains the materials in such manner as to encourage greater and easier access for the patron. Extensive use of the photocopy service within the Circulation Unit allows greater and more frequent use to be made of library literature. A considerable activity of this Unit is the interlibrary loan program, which makes available books and journals not found in the NIH Library but required by NIH scientists.

Another important readers' service offered is the translation of scientific literature. Most of the European languages are handled by staff translators. Other languages are translated by contractual arrangements.

Technical Services. Technical services include the acquisition of books, journals, and other printed materials needed to support the research efforts of NIH scientists and the processing of these materials for use. Continual review and revision of the records are needed to remain responsive to the changing needs of the scientists. A gift and exchange program permits valuable materials to be added to the collection at little cost to the Library.

Bibliographical Services. Although the concept of bibliographical support for the scientist through close liaison with the programs of the Institutes was approved in June 1963, this program has only been given gradual implementation. When it is fully implemented, the staff of the Bibliographical Services Section will assume full responsibility for these liaison functions. Also included will be programs of mechanized information handling and coordination with existing programs at the Division of Computer Research and Technology, National Library of Medicine, and other Government agencies.

C. Program Progress and Accomplishments

The second librarian internship program began on August 30, 1965, with the arrival of two interns from the University of Denver Library School and Catholic University Library School. They were given rotating work assignments in all units of the Library. The interns were provided an opportunity to gain work experience in librarianship and information handling while making a substantial contribution to the service program of the NIH Library. Both interns enrolled in the first year of the internship program remained with the Library as reference librarians.

Following completion of an automation feasibility study, it was decided to automate the business-like transactions of the Library Branch. Early in January 1966, a Systems Analyst was employed by the Library Branch to prepare the manual system for automation and design an adaptation of an IBM system prior to its implementation. The system being designed will

not be computer based initially. However, it is being designed with subsequent computer link-up in mind.

The planning phase of the new library facility was completed with the presentation of the final architectural drawings in January 1966. Planning also included the completion of written specifications covering the new equipment for the facility. Tentative plans were drawn for the physical transfer of staff, collection, and equipment. Construction was begun during late spring of 1966. Present plans call for completion of the new extension in August 1967.

An end product workload reporting system was installed in the Library Branch on March 1, 1966. The statistical reporting for the Branch for March was based on the new approach. Under the end product workload reporting system, the average processing time is the estimate for each end product, and the standard man-hours produced in an activity represent the overall estimate for that activity. The end product system can be used when the work processes are recurring and are relatively short-cycled.

Extensive in-house training of the NIH Library staff was conducted during FY 1966. Beginning November 1, 1965, two reference librarians were detailed to the National Library of Medicine's indexing and searching programs for four months. This training prepares reference librarians as formulators of Medlars search requests. On April 1, 1966, a third reference librarian entered the training program.

On November 23, 1965, the first of four in-library training lectures and demonstrations on laboratory techniques was given by an NIH scientist for translators. Subsequent lectures were attended by certain reference librarians. Proposed lectures will cover a wide variety of scientific subjects and will update the translating and reference personnel's familiarity with scientific terminology and research programs.

Phase II of Grid Management was held in the Library Branch from November 1-5, 1965. The Grid Management concept represents a new management technique that is expected to "provide a key for unraveling and solving managerial problems."

An in-house training program was held from December 9-22 for nonprofessional staff members of the Circulation Unit. Two-week exchanges of positions provided four participants the practical experience of performing duties ordinarily assigned to other staff members. It is expected that training of this type will help staff members develop greater overall library competence and will provide greater promotion opportunities for the participants.

Readers Services

Photocopying Service. Two Xerox 914 Photocopiers are used to fill urgent photocopy requests of 20 pages or less. Longer articles are photographed, processed, and then printed by the Xerox Copyflo. A new Recordak Camera, purchased and modified during the year, is used to photograph articles before they are run through the Xerox Copyflo.

Having the new camera enabled the Library to return equipment that had been borrowed from the National Library of Medicine for three years. The coupon identification system introduced in FY 1965 remained in effect during FY 1966.

Circulation. A total of 37,537 books and journals were circulated during FY 1966. This total represents a decrease of 442 volumes since last year. The decrease has resulted from the policy that now prohibits the circulation of materials for which only one copy is owned by the Library. A proportionate increase of photocopies made can also be attributed to this circulation policy.

During FY 1966, 4,666 volumes were loaned to other libraries and 9,736 volumes were borrowed from other libraries throughout the United States for NIH personnel. Translations loaned to libraries totaled 4,465 in FY 1966.

Reference Service. Experienced reference librarians with specialized science backgrounds are a part of the Library staff. Much emphasis is placed on supplying immediate answers to reference questions asked by phone or in person. Reference librarians regularly scan the current scientific literature to keep abreast of trends and developments in the sciences. The book selections program is an important activity shared by the reference librarians and a Selections Review Committee. Included in this program is a continuing search of journals, publishers' catalogs, national book catalogs, and announcements for current in-scope publications needed for the collection.

During the year the Selections Review Committee requested the assistance of scientists at NIH to evaluate the strength of different subject categories of scientific literature within the library collection. It is felt that a more valuable collection will result from the evaluations now being conducted by scientists who know well the subject categories being inspected.

Bibliographies requiring extensive searching were prepared by the Reference Unit on specific subjects requested by NIH investigators as were bibliographies of bibliographies, biobibliographies, and short reference lists. The total number of Medlars searches completed in FY 1966 was 154.

Translating. The NIH Translations Index, Supplement II, 1965 was compiled and distributed widely. The supplement again includes a subject index and a list of journal abbreviations. Each year approximately 500 articles are translated with an estimated 75 of these handled by contractors. Oral translations for the year consumed approximately 940 man-hours. Tape recordings were provided on request.

Technical Services

Acquisitions. During this 12-month reporting period, the Library's collection was strengthened by the addition of 5,247 new books and by

the receipt of 831 books through gift and exchange. For FY 1966, the Library placed subscriptions to more than 2,000 journal titles and received an additional 800 as gifts or on exchange.

In January 1966 the design of the automated system for all acquisitions and recording processes was begun. It is anticipated that the information now recorded on plain 3X5 index cards will be punched on IBM cards shortly after the close of the current fiscal year. A punched card system will allow information requested concerning book purchases to be made available much more quickly than is now the case.

Cataloging. Revision of the Periodical Record preparatory to its automation was completed. Staff shortages necessitated the handling of this project on an overtime basis.

Bibliographical Services

During January 1966, the Chief of the Bibliographical Services Section entered on duty. She became acquainted with the existing manual procedures within the Library Branch in preparation for her role in the design of an automation program. She has recommended alterations in the book selection procedure for the Library and has developed a plan for the systematic evaluation of the quality of the entire book and journal collection. An alerting service introduced by a former Liaison Officer assigned to NIAID was expanded to include not only bibliographies compiled by reference librarians but also pertinent Medlars searches and reprints. User studies are being planned to determine the extent to which certain subject groups of scientists are using the library services and the extent to which their needs are being met.

A proposal for a satellite library for the new Division of Computer Research and Technology was prepared by the Chief of Bibliographical Services at the request of the Chief, Library Branch and the Acting Chief of the new Division. It is expected that this proposal will serve as a prototype for future satellite libraries at NIH.

D. Problems

The recruitment of well-qualified persons for vacancies is difficult. Obstacles are encountered and too much time is spent attempting to employ and bring on board qualified candidates for key positions. Patience usually results in appointments being made but only after some candidates withdraw their names for consideration because of the excessive time factor.

The congested and poorly designed library space still causes the greatest problems. The present library is noisy and hard to supervise, and the security problem is difficult to handle. The majority of these space and security problems will be solved when the new library is completed.

E. Program Plans

Planning the move to new library facilities in the summer of 1967 has high

priority. Detailed specifications, bids, acquisition of new equipment, and plans for moving existing equipment and the library collection will receive additional attention.

Further implementation of the Bibliographical Services Section is planned.

A third medical library internship program conducted by the NIH Library is planned for 1966/67, beginning August 29. The two interns selected for the program will receive practical training at all levels while making contributions to the day-to-day operations of the Library.

The Library staff will continue to work closely with the Systems Analyst during the design phase of an automated system for the repetitive processes of the Library Branch. Implementation of the system will provide staff members an opportunity to develop expertise in programing for and understanding of the computer and its potential value to libraries.

Satellite libraries administratively tied to the NIH Library will be encouraged and developed in the Institutes/Divisions expressing interest in such an arrangement. It is felt that better organization of existing collections and proper development and supervision of new library collections within the Institutes/Divisions will result in more effective use.

F. Publications

Martin, J. A., et al: Twenty-five years of translating service at NIH. Bulletin of the Medical Library Association 53: 422-425, July 1965.

National Institutes of Health Library: NIH Library Booklist. Bethesda, Md., 1965, bimonthly.

National Institutes of Health Library: Periodicals Currently Received in the NIH Library, 1966. U. S. Dept. of Health, Education, and Welfare, Public Health Service Publ. No. 1313. Washington, D. C., U. S. Govt. Print. Off., 1966, 134 pp.

National Institutes of Health Library: Recent Translations, a Selected List. Bethesda, Md., 1966, monthly.

National Institutes of Health Library: Index of Translations, Supplement II. Washington, D. C., U. S. Govt. Print. Off., 1966, 92 pp.

Summary of Branch Activities July 1, 1965, through June 30, 1966

BIOMEDICAL ENGINEERING AND INSTRUMENTATION BRANCH

Dr. Lester Goodman
Branch Chief

I. SUMMARY

Effectiveness of the Biomedical Engineering and Instrumentation Branch professional and technical support provided to the NIH research program has been significantly increased by (1) an improved organization structure that permits better definition of functions, more efficient management, and enhanced utilization of research and development potential; (2) a distinct elevation in the level of professional staff; (3) the initiation of the "satellite" technical support unit concept; (4) improved workload and financial reporting with extension of automatic data processing; and (5) a vigorous professional and technical training program.

Engineering and technical talent has been better utilized in significant NIH research and development projects. Branch professionals have made extensive contributions to programs involving mechanization, automation, computation, physiological monitoring, artificial organs, surgical procedures, analytical instrumentation, and laser technology.

The technical service sections processed approximately 13,000 assignments, an increase of 11 percent over the preceding fiscal year, with no commensurate increase in staff.

Branch engineers were involved with approximately 400 different research and development projects and recorded more than 400 man-days of professional consultation with the scientific research staff of the Institutes.

The image of Biomedical Engineering and Instrumentation has been significantly enhanced locally within the NIH and broadly throughout the community at large.

II. BRANCH PROGRAMS

A. Objectives

To fulfill the need for engineering knowledge and technical skills in pursuit of health research at NIH.

To provide direct and consultative engineering support to clinical and biomedical research projects, including advice on systems analysis, experimental design, and synthesis of technical expedients.

To design, develop, fabricate, and evaluate special purpose devices and systems not commercially available.

To maintain and repair scientific laboratory and clinical equipment.

To provide communication between NIH and the community on engineering support to clinical and biomedical research.

To serve as a source of informed authority for the community in matters pertaining to technology and health.

B. Current Programs

The primary purpose of the Branch is to provide service and support to the entire intramural program of the NIH. The programs and activities, therefore, are identified with nearly all of the individual programs and activities that constitute the intramural research effort. The overall program is best distinguished by the coordinated effort of its operating units identified and characterized as follows:

1. Instrument Fabrication Section

a. Prepares minor designs of, makes major modifications to, and fabricates biomedical equipment and instrumentation systems.

b. Develops improved production techniques for the complete range of physical materials.

c. Operates facilities for the production of devices requiring special tools and skills in the electronic, electrical, glass, mechanical, optical, rubber, plastics, welding, and sheet metal categories.

2. Systems Maintenance Section

a. Maintains, repairs, and modifies to a limited degree biomedical equipment and instrumentation systems.

b. Instructs technicians and scientists in the proper use and operation of especially complex instruments and devices.

c. Operates facilities for the maintenance and repair of devices requiring special tools and skills in chemical, electrical, electronic, mechanical, and optical categories.

3. Electrical and Electronic Engineering Section

a. Provides direct and consultative electrical and electronic engineering support to clinical and biomedical research projects at the NIH.

b. Designs, develops, and evaluates electrical and electronic instrumentation and equipment used in research and clinical applications.

c. Provides communication between the NIH and the community on electrical and electronic engineering support to clinical and biomedical research.

4. Chemical Engineering Section

a. Provides direct and consultative chemical engineering support to clinical and biomedical research projects at the NIH.

b. Designs, develops, and evaluates chemical instrumentation and equipment used in research and clinical applications.

c. Provides communication between the NIH and the community on chemical engineering support to clinical and biomedical research.

5. Mechanical Engineering Section

a. Provides direct and consultative mechanical engineering support to clinical and biomedical research projects at NIH.

b. Designs, develops, and evaluates mechanical instrumentation and equipment used in research and clinical applications.

c. Provides communication between the NIH and the community on mechanical engineering support to clinical and biomedical research.

6. Branch Engineers

This small group of senior professionals serve as staff consultants in the Office of the Chief and are assigned to organize, conduct, and oversee particular comprehensive, long-range research and development projects. They provide unique scientific and engineering knowledge to the Branch and the NIH.

7. Supply

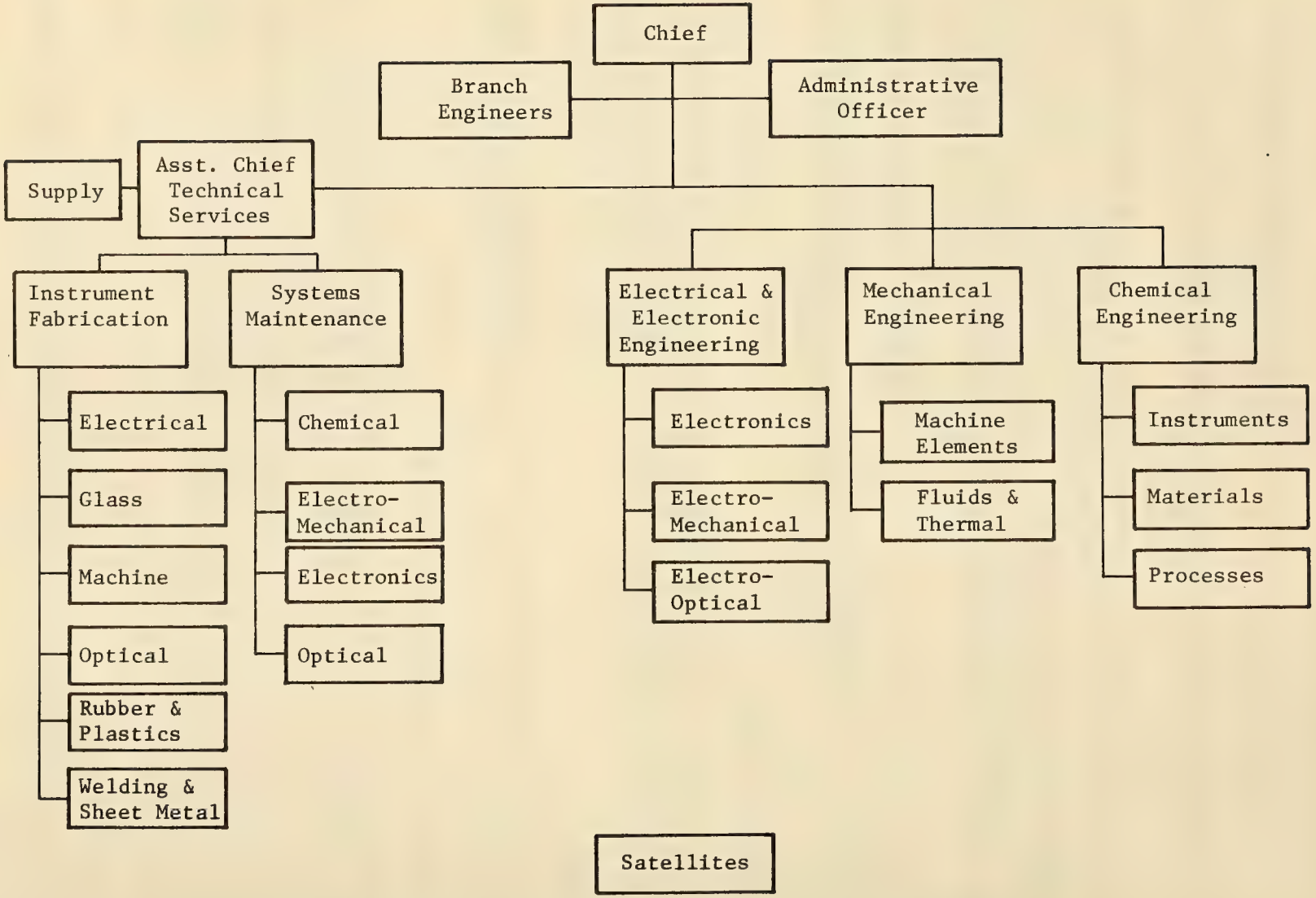
Provides for the acquisition and disposition of materials, parts, and equipment required for Branch operations. Maintains controlled inventory stocks and records.

C. Program Progress and Accomplishments

The effectiveness of professional and technical support by BEIB to the research program of NIH has been significantly increased. These improvements result directly from emphasis on the enhancement of the role of professional physical scientists and engineers as collaborators and prime contributors in health research rather than as subordinate service staff. This emphasis by management is generally acknowledged and supported by the scientific community of NIH. The entire staff of BEIB shares this improved status with the direct results of greater personal pride in the Branch, improved morale, increased productivity, and higher efficiency.

1. Reorganization

The Branch has been restructured as shown by the chart on the following page.



Biomedical Engineering and Instrumentation Branch

The former Engineering Development Section and Electronic Engineering Section are merged into the present Electrical and Electronic Engineering Section to provide better coordination of functions.

The Chemical Engineering Section is newly created in response to demonstrated needs of the research program in keeping with modern trends in the field of biomedical engineering.

The group of Branch Engineers is innovated to provide a means for senior professional staff with certain special knowledge and skills to pursue particular long-range research and development projects free from line responsibility.

The new post of Assistant Chief for Technical Services is primarily charged with the coordination of the Branch activities which fall under the Service and Supply Fund mechanism. These include the Instrument Fabrication and Systems Maintenance Sections together with the Supply Unit. Operating experience under the new organization clearly demonstrates marked improvement.

2. Satellites

The concept of "satellites" of BEIB has been activated. The "satellite" is a semi-autonomous technical support unit composed of engineers, technicians, and shop facilities located remotely from the central Branch. The satellite system places technical resources at close hand to the scientific staff. The group is immediately responsive to particular on-site program needs but is administratively responsible to BEIB management. The central Branch provides overall coordination and technical guidance to the satellite personnel, who are intimately familiar with the particular problems and personnel in their area. A full satellite is now in successful operation in the Clinical Center. Expansion of similar groups in NIDR and NCI is contemplated at an early date.

Plans for the Gerontology Branch, NICHD, in Baltimore and for the new NICHD construction in Bethesda include provision for satellites. The explicit mechanism for financing satellite operations remains undefined.

3. Space

A serious problem regarding adequate space for central Branch operations in Building 13 continues to exist. The situation is especially critical for the Systems Maintenance Section Shop and for the engineering staff. Overcrowding in these areas has reached the point of hazard, even though the number of personnel is significantly below current ceilings and expectations for the near future. It is anticipated that an expansion of about 5600 sq. ft. early in FY 1967 will alleviate the situation to a considerable extent. Plans have been completed for the expansion, and negotiations are under way.

A relatively minor expansion and alteration of the welding shop in the Instrument Fabrication area relieved an especially hazardous situation and improved the capability of the welding and sheet metal unit to process large structures.

4. Workload

The IFS processed approximately 4,300 different project assignments involving minor design, fabrication, or major modification. The SMS processed approximately 8,800 different project assignments involving maintenance, repair, or minor modification. The total, 13,100, represents approximately an 11 percent increase over FY 1965, with no commensurate increase in staff.

BEIB engineers were involved with approximately 400 different research and development projects and recorded over 400 man-days of professional consultation on a casual basis. A significant increase in the number of comprehensive long-range projects with which the Branch is involved is noted as an important characteristic of the evolution of the role of BEIB in the NIH program. Individual projects of special interest are detailed in section III below. Particular attention is called to significant contributions currently being made in the following:

a. Improvement in performance and utility of the physiological monitoring and display system in the surgical wing of the Clinical Center.

b. Systems design and construction for mechanization and automation of laboratory and clinical procedures.

c. Research and development in artificial organs and prostheses including studies of basic phenomena and construction of devices for artificial heart components and hemodyalysis therapy.

d. Systems design and construction of devices to gain more benefits from automatic data processing and electronic computation and control.

e. Improved methods and tools for cardiac and neurosurgery.

f. The development of the laser as a useful device in life science research and therapy, as well as extension of its physical potential.

g. The development of a surface tension apparatus. This project applies feedback control to surface tension measurements made with a Langmuir trough to study the effect of various chemical constituencies on the properties of surface films. Two modes of operation are possible: constant area with tension recorded as a function of time, or constant tension with area recorded as a function of time. The system permits automatic measurement of the surface tension of a film which is involved in a chemical reaction, with a high degree of accuracy and convenience.

It is important to note that these accomplishments are not the sole products of the professional staff of BEIB. They represent the collaborative effort of the scientific staff of the NIH together with BEIB's engineers. Further, they would not exist were it not for the full participation and superior skills of the technical service sections.

Significant improvements in workload reporting have been accomplished. All operating units of the Branch now are included in a uniform manner. The instrument is not yet completely satisfactory but is evolving as time and experience accrue. The more comprehensive methods provide important information, heretofore unobtainable, for improved operations analysis and forecasting.

5. Engineering Advances

BEIB has designed and developed a considerable number of special purpose devices in support of Institute research projects. Unique items, of more than casual interest, are identified as follows:

A special low noise thermocouple amplifier measures temperature changes in the heart during a cycle of operation.

A projector timer permits programing of two projection intervals over a 12-event sequence of a neurological operation. It includes stimulus triggers and event mark voltages for analog recording.

A 24-channel analog temperature recording system is used in low temperature neurological temperature gradient studies.

A radio frequency excited gas ionization apparatus removes organic material from bone samples without destroying the crystalline structure.

A solid state precision temperature control system is used in a bench-type centrifuge.

An automatic serial diluting machine provides for rapid sterilization of the lips of glass tubes and subsequent injection of media or reagents into tubes arranged in racks of 64 each to reduce manual handling. A conveyor system sequentially transfers the racks to individual treatment stations where 16 tubes in a single row are treated simultaneously.

A blood vessel stapling machine staples fascia into artificial arteries for humans. Staples 0.1" long made of 0.01" diameter tantalum wire and formed to prevent complete closing, avoid tissue damage.

A dual stepping motor assembly controls wavelength and slit width of a monochromator. Motor drive is electronically controlled by outputs from a LINC computer. A log ratio converter accepts inputs from a reference and a signal photomultiplier tube and provides a suitably conditioned signal to the LINC computer.

A random pulse generator drives a photic stimulator, part of a response averaging instrumentation system. An aperiodic stimulation is required to preclude the subject's anticipating the stimulus.

An electronic automatic scoring board scores the patient during psychophysiological testing, reducing the time required to check the test results and increasing their accuracy.

A glass knife scoring device holds and scores a sheet of glass to permit controlled manufacture of microtome knives.

A special purpose analog computer for cardiac surgery permits on-line and off-line computation of various cardiac functions observed during open heart surgery.

A digital processing unit performs threshold detection, base line stabilization, and peak detection. The unit employs a unique microcircuit bidirectional counter and has been integrated with a chromatograph data acquisition system.

A fully automated dynamic surface tension measuring apparatus features constant force or constant.area operating modes.

A special purpose computer determines the average resting potential of neurons as they are pierced by the insertion of a microelectrode in the brain.

A digital data acquisition system expedites protein diffraction studies.

An electronic control unit controls a vacuum heart booster and synchronizes it with the electrocardiogram.

Electronic circuitry to permit remote monitoring of an electromagnetic blood flowmeter has been installed in the cardiac surgery.

A special microelectrode recording console handles a multiplicity of EEG channels in the neurology operating room.

A photocopy device for use of the NIH Library produces rapid microfilm copy from books and bound volumes of journals. Semiautomatic and self-adjusting, it accepts volumes of random size, thickness, and page progression.

An acid analyzer high-pressure manifold withstands pressures up to 400 psi and provides considerable utility for programing analyzers for automatic multiple runs.

A spectrophotometer power supply is being substituted for storage batteries in equipment used for visible wavelength spectrophotometry. Two hundred units have been installed at a savings of $463 per unit, with a net savings of $92,600.

An ultracentrifuge vacuum detector has been modified to shut down the ultracentrifuge when there is a loss of vacuum. Inadequate vacuum causes excessive rotor heating and destroys samples.

A high-speed, low noise pH meter permits monitoring of fast enzyme reactions.

A multiple filter holder provides a six-fold increase in the rate with which samples of labelled proteins and polynucleotides are prepared for analysis.

An angulated implant electrode hydraulic microdrive can be so positioned as to penetrate any point beneath the skull, and the point of penetration can be accurately known by means of calibrations on an adapter.

An automated ergograph provides electro-pneumatically programmed forces to a squeeze grip operated by a monkey. One hundred force levels, within the range of 0 to 15 pounds, are produced, depending upon the monkey's success at closure. Digital and analog readouts are available for eventual automatic computer processing of data records to expedite psychophysiological studies.

6. Inventory

Improvements in inventory control with more use of automatic data processing have been achieved. An economic order quantity system is in development.

7. Service Contracts

The Branch encourages the use of contractual services with special emphasis on service-type contracts with certain scientific equipment suppliers. For the past two years, a fixed price contract with Beckman Corporation, Spinco Division, has proven most beneficial to the scientific staff. Specific benefits derived include:

a. Consulting services on custom engineering modifications and special accessories.

b. Providing information for researchers and technicians on new techniques and applications as developed.

c. Direct liaison with NIH researchers and Branch personnel with the research, application, engineering, and marketing departments.

d. Guidance and assistance in setting up scheduled service and maintenance programs.

A training seminar was conducted by Beckman in January and February on molecular weight determinations through the use of ultracentrifugation. Four one-week classes were held, with a total of 56 researchers participating. Ninety applications were received, but because of lack of space and time, the entire group could not be accommodated.

Negotiations for an additional similar contract with the Beckman Special Process Instrument Division is under way for spectrophotometers, pH meters, and gas chromatographic and liquid scintillator counters.

8. Utilization of Scientific Equipment

An acceptable overall economic equipment utilization program for NIH is sorely needed. The Branch maintains a small informal loan program initiated a year ago. The turnover value of equipment loaned to NIH researchers this year was $120,000 compared to $50,000 for the previous year. In addition, the Branch

intends to initiate an inspection program, in cooperation with SMB, concerned with serviceability, utility, and condition of all scientific equipment passing through surplus property.

9. Service and Supply Fund Operations Report

An improved data processing reporting system was programmed during the year. This new system has improved our transactions with the Institutes by providing them with timely, up-to-date reports of costs of projects completed and in process.

A marked reduction of paper work and time delay was achieved for maintenance and repair tasks by establishing a basic monthly obligation under a single assigned allotment number and project number for each program. Similarly, savings have been achieved by increasing authority for processing of fabrication tasks to $200 without requiring formal administrative clearance.

10. Training

A vigorous professional and technical training program is emphasized to provide improved, up-to-date support to NIH in engineering, fabrication, and maintenance. Twenty-six employees participated in academic and technical courses. Sixteen (215 man-days) attended formal education and training courses. Fifteen (120 man-days) received specialized training on scientific equipment at suppliers' plants and at the NIH.

D. Problems

1. Technical Personnel

The abolishment of the Instrument Making Series by the Civil Service Commission has created difficulties in the SMS. A large number of the personnel have been reclassified in Wage Board positions under the title of Medical Equipment Repairers. Since no open examinations exist at present, recruiting and retention in this area is difficult, with most new appointees receiving taper appointments. It appears that January 1967 will be the earliest date examinations will be announced, if at all, because of consolidation of the Civil Service Examining Boards. The Personnel Management Branch is seeking means by which career conversions can be realized and other recruitment sources exploited.

2. Professional Personnel

It remains difficult to recruit and retain the type of high level professionals required to meet current demands and to be prepared for future expansion of the NIH program. Salaries and advancement opportunities at the lower grades for recent baccalaureates remain slightly lower than equivalent opportunities in industry. Beginning salaries are on the order of 5 to 10 percent low. The disparity increases progressively as grade levels increase, so that at the upper grades of GS-13, 14, and 15, industry and academic institutions

offer up to 50 percent more than Civil Service. In addition, advancement opportunities are severely limited at the upper grades. The problem is aggravated by the fact that other Government agencies and even other divisions within NIH are able to offer higher compensation than can DRS. It has been recognized by higher authority that a more liberal policy is essential; however, substantive progress seems to be very slow in appearing.

Modernization of the traditional policies of the Civil Service Commission and personnel officers with regard to all personnel actions would seem appropriate in this era of rapid growth and change. Major modifications are essential if the full potential for contribution is to be realized.

3. Space

The problem of inadequate space for the central Branch in Building 13 appears to be on the way to partial solution in FY 1967, since plans for expansion have been initiated. At the anticipated rate of growth, further enlargement will be required two to four years hence.

As the satellite concept becomes more fully developed, provisions for more adequate space will probably be required in the Clinical Center, Building 30, and Building 6.

Should an overall economic equipment utilization program be developed for all of NIH, more space will be required in Building 13.

4. Large-scale Development Programs

Concern is expressed over the proper allocation and management of engineering and technical responsibilities vis-a-vis large-scale development programs such as the Artificial Heart Systems Development Program and the Artificial Kidney-Hemodyalysis Program. It is hoped that NIH will fulfill its technological responsibilities for these programs as well as the medical aspects for which it is uniquely qualified.

5. Program Conflicts

The demand for close engineering and technical support in the performance of health research is growing very rapidly. A result is conflict over the degree to which identification and decentralization of support facilities are desirable. There are advantages to each Institute/Division having its own captive technical support team. There are advantages in having a single comprehensive central organization. Multiplication of facilities and functions can be extremely inefficient. Great care must be taken in seeking to achieve the most effective distribution of facilities and personnel. This is especially important where personnel, physical, and financial resources are limited.

E. Program Plans

As indicated in the preceding sections, the Branch has initiated a number of far-reaching programs and has identified a number of deep-rooted significant

problems. Explicit plans for the immediate future are restricted to the rapid enhancement of established programs and aggressive pursuit of solutions to the problems stated. An important consideration in this regard includes an anticipated growth rate of 15 percent per year, together with the observation that the professional and technical resources of BEIB are not adequate to meet current demands in an adequate fashion.

F. Publications, Presentations, Patents, and Awards

1. Publications

Alt, F., Harlow, W. G., and Metz, H. D.: Biomechanical instrumentation engineering. In "Biomechanics and Related Bio-engineering Topics," Proceedings of a symposium held in Glasgow, September 1964. New York, N. Y., Pergamon Press, 1965, pp. 9-19. (Note: Dr. Alt and Mr. Harlow are no longer with NIH.)

Cohen, G. S.: An instrumentation system for surgical neurology. In Iwai, Y. (Ed.): Digest of the 6th International Conference on Medical Electronics and Biological Engineering, August 22-27, 1965, Tokyo, Japan, pp. 364-365. (Japan Society of Medical Electronics and Biological Engineering) (Presented in Tokyo, Japan, Aug. 1965.)

_____: Design of the operating room. In Segal, Bernard L. (Ed.): Engineering in the Practice of Medicine. Baltimore, Md., Williams and Wilkins Publishing Co. To be published spring of 1966. (Presented at the "Engineering in the Practice of Medicine" meeting sponsored by the Dept. of Medicine of the Hahnemann Medical College and Hospital and the Group for Engineering in Medicine and Biology of the Philadelphia Section of IEEE, Philadelphia, Pa., Nov. 1965.)

Friauf, W. S.: Uses of integrated circuits in biomedical engineering.
Harlow, W. G.: An automatic microtitration system for viral serological investigations.
Metz, H. D.: The design of an artificial kidney center.
Riggle, G. C., Anderson, F. O., and Chalifoux, H. D.: The tetrascanner: a new device for detecting brain tumors. In Frommer, P. L. and Vurek, G. G. (Eds.): Proceedings of the 18th Annual Conference on Engineering in Medicine and Biology 1965. Philadelphia, Pa., The Conference Committee for the 18th Annual Conference on Engineering in Medicine and Biology, Herman P. Schwan, Chairman, Moore School of Electrical Engineering, University of Pennsylvania, Philadelphia, Pa., 1965, Vol. 7, pp. 216, 181, 50, 13, respectively. (Papers presented in Philadelphia, Pa., Nov. 1965.)

Goodman, L.: Biomedical engineering and instrumentation. Paper No. 66-MD-5. The American Society of Mechanical Engineers, United Engineering Center, 345 East 47th Street, New York, N. Y. 10017 (Presented at the ASME Design Engineering Conference, Chicago, Ill., May 1966.)

2. Presentations

Cohen, G. S.: Biomedical engineering and instrumentation branch support. Chesapeake Area of Hospital Engineers, Bethesda, Md., March 1966.

_____ and Goodman, L.: Engineering in medicine. Delaware Hospital Association, Wilmington, Del., Jan. 1966.

Goodman, L.: Engineering in medicine. Potomac Chapter, Maryland Society of Engineers, Langley Park, Md., Nov. 1965.

_____: Biomedical engineering at the National Institutes of Health. University of New Hampshire, Durham, N. H., March 1966.

_____: Federal support in biomechanics. Panel presentation. First ASME Human Factors Conference, Washington, D. C., March 1966.

_____: Biomedical engineering and instrumentation at the National Institutes of Health. Instrument Society of America, Philadelphia Section, Philadelphia, Pa., May 1966.

Simon, G. A.: Ilustrated lecture describing the role of BEIB in the mission of NIH. One of a series of orientation lectures for PHS Commissioned Officers at the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, Aug. 1965.

3. Patents

Metz, H. D.: Microtome section pickup and manipulator. Notice of Allowance and Issue Fee dated April 6, 1966.

Leder, P. and Byrne, C. J.: (Patent applied for) Multiple Filtration Apparatus, patent application 404-993, filed October 19, 1964.

4. Awards

Mr. John M. F. DeBroske received a cash award for "Superior Performance by serving in dual capacity as Chief, Instrument Fabrication Section, and full assistant to the Acting Chief on day-to-day operations and overall administration of the Biomedical Engineering and Instrumentation Branch." December 1965.

Mr. Walter S. Friauf received a quality increase for his level of proficiency in solid state circuit design. January 1966.

Mr. William H. Schuette received a quality increase for his exceptional ingenuity and productivity in developing devices and systems used in NIH laboratories and clinics. May 1966.

Serial No. DRS-BEIB-1 (c)

1. Biomedical Engineering and Instrumentation Branch
2. Mechanical Engineering Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Artificial Kidney Center

Previous Serial Number: None

Principal Investigators: E. A. Gombos, Howard D. Metz

Other Investigator: Homer D. Chalifoux

Cooperating Unit: Nephrology Laboratory, Veterans Administration Hospital

Man-years:

Total:	1.75
Professional:	1.0
Others:	.75

Project Description:

Objective: To develop and equip a hemodyalysis treatment center serving 10 patients simultaneously.

The center is nearing completion at the Veterans Administration Hospital in Washington, D. C. It includes a central dialysate preparation system which feeds individual Kiel membrane bedside units. The central system prepares, monitors, and pumps dialysate to each bedside kidney. Temperatures, pressures, and flow rates are monitored and regulated at the bedside for each patient. Safety devices within the central system assure proper concentration and temperature, or, in case of malfunction, stop the dialysate flow, signal an alarm, and indicate the cause. To prevent bacterial growth, the system incorporates means for automatic cleaning and sterilizing.

Publication:

Metz, H. D.: The design of an artificial kidney center. In Frommer, P. L. and Vurek, G. G. (Eds.): Proceedings of the 18th Annual Conference on Engineering in Medicine and Biology 1965. Philadelphia, Pa., The Conference Committee for the 18th Annual Conference on Engineering in Medicine and Biology, Herman P. Schwan, Philadelphia, Pa., 1965, Vol. 7, p. 50.

Serial No. DRS-BEIB-2

1. Biomedical Engineering and Instrumentation Branch
2. Mechanical Engineering Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Automatic Microtitration Study

Previous Serial Number: None

Principal Investigators: Winton G. Harlow (no longer at NIH)
George A. Simon

Other Investigator: George L. Lawrence

Cooperating Unit: Perinatal Research Branch, NINDB

Man-years:

Total:	2.0
Professional:	1.5
Others:	.5

Project Description:

Objective: To achieve an integrated, automated system for bulk processing of microtitration work. The study consists of four main projects, as follows:

1. A diluting machine for automatically performing accurate serial dilutions in rows of microtitration cups.

2. An injection machine for preparation of microtitration cups, in which accurately measured droplets of reagent are rapidly injected into sets of cups.

3. An injection machine for mass preparation of racks of test tubes. Each tube is flame-sterilized before and after injection of measured quantities of nutrient media.

4. A data-taking, readout device to evaluate the amount of precipitate formed in the bottom of titration cups. An optical system capable of measuring the quantity of hemoglobin precipitated from lysed blood cells.

These studies have included experimental optical systems, high-speed photography studies of droplet formation at jet orifices, transfer of precise quantities of liquids by means of wire loops, and maintenance of sterility by various sealing materials.

Publication:

Harlow, W. G.: An automatic microtitration system for viral serological investigations. In Frommer, P. L. and Vurek, G. G. (Eds.): Proceedings of the 18th Annual Conference on Engineering in Medicine and Biology 1965. Philadelphia, Pa., The Conference Committee for the 18th Annual Conference on Engineering in Medicine and Biology, Herman P. Schwan, Philadelphia, Pa., 1965, Vol. 7, p. 181.

Serial No. DRS-BEIB-3

1. Biomedical Engineering and Instrumentation Branch
2. Mechanical Engineering Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Heart Assist Pump

Previous Serial Number: None

Principal Investigators: Howard D. Metz, William S. Pierce

Other Investigators: John W. Boretos, John L. Fogle

Cooperating Unit: Surgery Branch, NHI

Man-years:

Total:	1.25
Professional:	1.0
Others:	.25

Project Description:

Objective: To construct a roller-type pump which can be placed in the human body as a permanent left ventricular heart-assist for patients requiring such partial relief. The current phase of this project is primarily concerned with engineering design and materials.

Methods Employed: The project is divided into three parts:

1. The development of a reliable, electrically powered implantable pump of an acceptable size, made of physiologically compatible materials, capable of handling the required blood flow rates.

2. The development of physiologically acceptable internal tubing material capable of withstanding long-term mechanical stresses for at least two years to failure.

3. The development of a measurement and control system to maintain correct pressure within the left atrium of the heart.

During the approximately one and one-fourth man-years devoted to this project thus far, one roller-type tester has been built. Information gained from this has led to the construction of a first prototype pump. Approximately two dozen types of tubing have been procured, or fabricated, and tested. First results indicate that a new properly balanced formulation of ethylene propylene terpolymer elastomer may be acceptable.

Serial No. DRS-BEIB-4

1. Biomedical Engineering and Instrumentation Branch
2. Mechanical Engineering Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Shock Damage Instrumentation

Previous Serial Number: None

Principal Investigators: Winton G. Harlow (no longer at NIH)
Ayub K. Ommaya

Other Investigators: George F. Norris, George A. Simon

Cooperating Unit: Surgical Neurology Branch, NINDB

Man-years:

Total:	1.0
Professional:	.75
Others:	.25

Project Description:

Objective: To provide means for acquiring quantitative measures of shock damage to nerve tissue through studies of injuries to the head and spinal column caused by whiplash.

Methods Employed: The Head Injury Study concerns shock damage to cranial tissue. Controlled mechanical shocks are administered to selected spots on the head of a test monkey by means of a bumper driven by an air cylinder. A restraint is provided by a bucket seat mounted on a universal pivoting system. Data are gathered by appropriate transducers and high-speed photography.

The Whiplash Study evaluates the effects of sudden accelerations on the upper portion of the spinal column. A restraint device has been adapted to a vehicle which travels along a 12-foot track at selected and controllable accelerations. Transducers attached to the vehicle and the test animal monitor forces, accelerations, and pressures. High-speed photography provides a permanent record of whiplash curves of the spinal column which can be related to subsequent clinical records.

Serial No. DRS-BEIB-5

1. Biomedical Engineering and Instrumentation Branch
2. Office of the Chief
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Utility of the Laser in Biological Research and Clinical Applications

Previous Serial Number: None

Principal Investigators: Grant C. Riggle, Alfred S. Ketcham

Other Investigators: Woodrow F. Stotler, Robert C. Hoye

Cooperating Unit: Surgery Branch, NCI

Man-years:

Total:	1.5
Professional:	.75
Others:	.75

Project Description:

Objective: To quantify the effects of laser energy as a surgical and experimental tool in several fields of medicine, including cancer and neurology.

Methods Employed: Utilize available commercial lasers; develop special units incorporating frequency multiplication; perform comparative studies on experimental animals to determine manifestations of various laser energy levels; develop instrumentation for control and measurement of laser performance.

Major Findings: Since program was newly initiated this fiscal year, it is too early to state definitive results.

Significance to Biomedical Research and the Program of the Institute: The potential benefits of a new type of energy for certain types of medical applications. The need for more complete understanding of the properties of laser power, its measurement and control.

Proposed Course of Project:

1. Development of accurate and precise methods for controlling and measuring laser energy. Corresponding development of new instrumentation which may not be commercially available.

2. Study of effects of various laser light parameters on tissue, such as frequency pulse width, height, waveforms, and optical patterns. Selection of optical materials for particular purposes.

3. Development and test of materials and operations standards for personnel safety.

Serial No. DRS-BEIB-6

1. Biomedical Engineering and Instrumentation Branch
2. Office of the Chief
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Automated Sample Combustion Development

Previous Serial Number: None

Principal Investigators: John I. Peterson, Seymour M. Perry

Other Investigator: Sidney Siegel

Cooperating Unit: Medicine Branch, NCI

Man-years:

Total:	.1
Professional:	.1
Others:	

Project Description:

Objective: Automation of the combustion technique for preparation of samples for radiotracer scintillation analysis.

Methods Employed: Experimental combustion techniques.

Major Findings: None to date.

Significance to Biomedical Research and the Program of the Institute: This development would reduce the cost and labor involved in the preparation of large numbers of samples.

Proposed Course of Project: The feasibility of a continuous flow combustion will be tested.

Serial No. DRS-BEIB-7 (c)

1. Biomedical Engineering and Instrumentation Branch
2. Office of the Chief
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Automated Urinalysis Development

Previous Serial Number: None

Principal Investigators: John I. Peterson, Donald Young

Other Investigators: None

Cooperating Unit: Clinical Pathology Department, CC

Man-years:

Total:	.3
Professional:	.3
Others:	

Project Description:

Objective: The development of methods and instrumentation to provide automatic analysis of urine samples, in a continuous operation, for several components of clinical significance.

Methods Employed: Mechanical engineering design and analytical methodology appropriate to the determinations of interest; namely, color, pH, specific gravity, glucose, hemoglobin, protein, ketones, bilirubin and urobilinogen.

Major Findings: None to date.

Significance to Biomedical Research and the Program of the Institute: The development of this equipment will facilitate screening of patients' urine on a routine basis, will provide better data than is available by dipstick tests, and will allow laboratory personnel to be freed from routine work for assignment to other development programs.

Proposed Course of Project: Concurrent development of mechanical handling techniques and adaptation and testing of analytical methods.

Serial No. DRS-BEIB-8 (c)

1. Biomedical Engineering and Instrumentation Branch
2. Office of the Chief
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Microbiology Automation

Previous Serial Number: None

Principal Investigators: John I. Peterson, Viola Mae Young

Other Investigators: None

Cooperating Unit: Clinical Pathology Department, CC

Man-years:

Total:	.1
Professional:	.1
Others:	

Project Description:

Objective: Development of a program for the automation of various aspects of clinical microbiology.

Methods Employed: Discussions between the Microbiology Service and BEIB personnel, to develop proposals for the program.

Major Findings: None to date.

Significance to Biomedical Research and the Program of the Institute: Automation of microbiology operation is aimed toward minimizing the labor and time delay involved in providing microbiological data which is critical to patients' therapy.

Proposed Course of Project: The development of primarily mechanical handling systems.

Serial No. DRS-BEIB-9

1. Biomedical Engineering and Instrumentation Branch
2. Office of the Chief
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: Prevention of Thrombosis on Intravascular Prostheses

Previous Serial Number: None

Principal Investigators: John I. Peterson, Nina Starr Braunwald

Other Investigator: Lawrence I. Bonchek

Cooperating Unit: Surgery Branch, NHI

Man-years:

Total:	.2
Professional:	.2
Others:	

Project Description:

Objective: To solve the problem of clot formation on implanted intravascular prostheses.

Methods Employed: The hypothesis, that coagulation can be prevented by an appropriate electrical charge applied to the prostheses, is being tested.

Major Findings: None to date.

Significance to Biomedical Research and the Program of the Institute: The coagulation problem is presently the greatest obstacle to successful use of implanted valves. The solution of this problem is a basic requirement of artificial heart development.

Proposed Course of Project: Electrical measurements are being made to establish the conditions which will prevent coagulation on test wires implanted in dogs.

Summary of Branch Activities July 1, 1965, through June 30, 1966

MEDICAL ARTS AND PHOTOGRAPHY BRANCH

Malcolm S. Ferguson
Branch Chief

I. SUMMARY

The Medical Arts and Photography Branch is continually striving to provide NIH scientists with the best available techniques of art and photography in support of their clinical and laboratory research. Thus, the quality, quantity, and promptness with which MAPB personnel respond to work requests from the I/D's are subjects of continuing concern. Training is also an activity receiving year-round attention. Instruction was received by several persons at nearby schools. Other training was received by MAPB employees at factory installations and at institutes operated by professional groups. Additionally, special seminars dealing with such topics as color--its photography and duplication, legibility, and animation techniques were held "in house" for selected employees.

Since MAPB was reorganized a year ago, benefits have been derived from the clarification of administrative responsibilities and the stimulation some employees received from managerial recognition. Increased production in some activities is conspicuous. The centralization of purchasing and travel responsibilities has resulted in better budget information as well as tighter material and equipment management.

Medical and general art services were sharpened by organizational and policy changes. During the year a work-performance record was set by the Drafting Unit. Unusual pieces of medical art were drawings of the developmental stages of four newly-discovered simian malaria parasites, models of arthritically crippled hands and feet, a model of the human eye, and a new rat cranial atlas. Work on publications and exhibits design continued in general illustration. One 1966 exhibit has been requested for long-term showing in the Smithsonian Institution. Design effort for exhibits is being channeled toward light-weight, sturdy forms which require less setup time and shipping protection.

The reorganization of the photographic services makes possible the immediate recognition of any lag in production. Past backlogs are gone. Trials are being made of roll film instead of cut film for use in patient and other clinical and scientific photography. The roll film is less expensive to use. There is a slight trend toward an increasing use of 2" x 2" slides over the older heavy $3\frac{1}{4}$"x 4" size.

Requests for motion picture work increased, especially for films of the "research report" variety. Filming in support of clinical studies and laboratory research has continued. MAPB capability in animation has increased, and there are prospects for its improvement in FY 1967.

A. Objectives

The objective of MAPB is to support the research programs of NIH with visual records of the clinical and biological problems and other phenomena which are being studied, and to complement the verbal dissemination of scientific information to both professional and public groups through associated photographs, motion pictures, and illustrations.

B. Current Programs

The programs of the Branch reach across a wide variety of art and photographic services: still and motion picture photography of clinical and laboratory research involving humans, animals, and related biological and physical material; photomacrography and photomicrography; public information photography; art work concerned with clinical illustration, graphics, statistical drafting, general illustration, exhibits design, moulage, and model fabrication; and cinematography including normal and high-speed camera work, editing, animation, and motion analysis.

During FY 1966, in addition to routine motion picture work, (i.e. patient and animal motility studies, interview and surgical operation recording, records of laboratory procedures, and the application of cinematography to research) several "scripted" research report films were completed, e.g. "Technique of Platelet Transfusion" for the Acute Leukemia Task Force, NCI; "Birth Defects: A Research Report" for NINDB; and "Pathogen-Free Animals" for the Laboratory Aids Branch, DRS. A film on the development of the NIAID vaccine program is underway.

Currently the photography service is trying out roll film instead of cut film for taking pictures of patients and for certain other clinical uses. It appears that in many instances the 35mm and 120-size roll color film can be used very satisfactorily. Roll film is much more economical both as to film stock and time required to handle and develop.

Two years' experience with a new work request form and reporting system has confirmed that the system is too expensive to use. Excessive time has to be taken to fill out the form, and the cost of translating the data to punch cards for data processing is high. During the past year a work sampling study was made under the supervision of a management analyst. The study is part of a Division-wide program to find an efficient way to record and report work accomplished. The information obtained will enable the Branch to project its budgetary requirements more realistically.

The responsibility of MAPB regarding exhibits is gradually changing. At present MAPB does the design and oversees the contracting and construction of exhibits and keeps the historical record of exhibits for the Institutes and Divisions. Its work on repairing exhibits is limited to "cosmetic" touches. MAPB is no longer concerned with the warehousing, shipping, set-up, striking, receiving, and extensive repairing of exhibits.

C. Program Progress and Accomplishments

Activities of the medical illustration service have resulted in several items worthy of attention. Drafting personnel have compiled a remarkable record of production. The number of completed charts and graphs exceeds by 30 percent the average production of the past four years. The number of employees has held steady, overtime is lower than it has been for eight years, and the character of the charts and graphs produced is unchanged. Response of the group to the challenge of increasing work plus a managerial change are probable causes for the increase in production.

Field work of the Laboratory of Parasitic Diseases, NIAID, has disclosed the existence of hitherto unknown simian species of malaria. Each of the developmental stages of four new species has been painted in color by a medical artist. In all, 25 to 30 illustrations were made for each species, and these are to be published in a monograph.

Plastic materials have been used for several years to make "to size" casts of the hands and feet of patients afflicted with arthritis. Making a series of these casts results in reproductions which show step-by-step the pathological change which takes place. This activity was revived during the year after a lapse of several months due to the death of the principal scientist who formerly requested these services.

An "Atlas of Cranio Facial Development in the Rat" has been completed during the year. The medical art service contributed all of the 50 illustrations.

A new eye fundus diagram chart was designed for use by the Ophthalmology Branch, NINDB. The charts permit the observer to record consistently and proportionally what is seen in the eye. Thus, by comparison of an early diagram with a recent one, changes are readily noted.

Another important project now under development is a life-size model of the human eye for NINDB. Technical problems were encountered when simulations of the cornea, iris, and retinal vessels were attempted. These difficulties have been overcome, and it is expected the model will have great instructional value.

The general illustration services continued to be active in the layout and design of NIH biomedical publications. Among the more important ones completed were: A Gift of Time, Cystic Fibrosis, For Your Information, Human Growth Hormone, Peptic Ulcer, Project Patients Must Be Fit, Psoriasis, Sickle Cell Anemia, Special Research Resources For The Biomedical Sciences, and Systemic Lupus Erythematosus.

During the report period, 24 exhibits were designed and 21 were revised and repaired. NIH exhibits have been on display at many important biomedical meetings in the U.S.A. and some at foreign meetings.

Design attention was given to constructing light-weight, easily-set-up exhibits. Using aluminum frames and 4' x 5' sheets of foam plastic cored material, a special four-panel exhibit was made and used on a trial basis for NIDR. Another NIDR exhibit, "Facts About Fluoridation", is being made to Smithsonian specifications for inclusion in the Institution's Hall of Medicine for long-term display.

MAPB artists have gained much valuable experience from several complex animation sequence assignments and have demonstrated increasing proficiency with this type of medium. However, having no formal training in animation, the artists encounter some problems with the more sophisticated types of presentation. This need has led to an arrangement whereby one artist is receiving apprentice experience in animation production at a recognized film studio.

The reorganization of the Photography Section is a little over one year old. Responsibility for different kinds of photographic work and the administration of the Section have been sharply delineated, and this has resulted in greater efficiency. Since the reorganization last year each position description in the Section has been updated.

Labor-saving equipment put into operation last year continues to yield dividends. Line copy negatives are all developed by automated equipment. About 80 percent of the continuous tone emulsions are also developed by the automatic processor. Negatives from "one time only" photo situations and all those exposures where the standard emulsion speed of the film is being modified require custom development. These cases make up the remaining 20 percent of the continuous tone materials handled in the laboratory.

There is a continuing trend on the part of MAPB clients toward greater dependence on the 2" x 2" slide. This size is not only more easily stored and transported, but it costs much less to produce than the older heavy glass $3\frac{1}{4}$" x 4" slide. In a recent month, 3,500 2" x 2" slides were produced, compared to 1,400 of the $3\frac{1}{4}$" x 4" size.

Pictures of NIH research activities taken by MAPB photographers have been requested and published in many well-known magazines during the year. Examples are: World Health, Medical World News, Newsweek, National Observer, and the New York Times magazine. Slide sets made for training and other purposes covered such subjects as procedures for food handlers in the Clinical Center, the nurse recruiting program, and the nursing facilities and out-patient area of the Clinical Center.

The principal part of the photographic work done by MAPB is in support of on-going research programs of NIH scientists. Sometimes this work has a consultive aspect. Several laboratories have acquired their own photomicrographic equipment. MAPB personnel are frequently asked to demonstrate the steps in calibrating the equipment, to test for optical alignment, and to show how the equipment is used to take photomicrographs. Other consultive services involve advice on the custom development of photographic emulsions where stated times, temperatures, and chemicals are varied to achieve a specific result.

A different kind of photographic service was given to the Division of Research Facilities and Resources. The ID photo booth was moved to the Westwood building and in two days all employees were photographed. Personnel lost very little time, and only one photographer was required to keep the photo booth supplied with chemicals and photographic paper. In addition, all NIH Commissioned Officer personnel (700) were provided with new identification photographs with a minimum of expense and no interference to routine photographic activities.

The motion picture activities of the Branch were organized into a Section in January 1965. Since that time the staff has gained experience in working as a team. An operator for the animation camera equipment has been trained through observation of operations in outside motion picture firms and through association on the job with a consultant animation cameraman. The motion picture personnel and artists concerned with animation production have also had the benefit of seminars presented by an animation director, a color motion picture film expert, and a medical communications specialist.

In addition to the "scripted" motion pictures, about 30 research-related projects of varying length and complexity were completed during the year. Some subjects covered dealt with: techniques in plaster limb casting, high-speed camera studies of the effect of a liquid being injected into another liquid and the turbulence in liquids passing through artificial heart valves, and the behavior of infant hamsters with neurological lesions. Motion picture coverage of the response to therapy of patients continued to be a service that was regularly requested by clinical investigators. All cinemicrography done by the Branch is performed in the Photography Section, which has the requisite microscope and associated equipment.

Editorial equipment of the Motion Picture Section is being upgraded, and a special editing area has been remodeled. This will provide a cleaner working environment and one that is less busy and noisy so that sound editing can be accomplished properly. In addition to the editing of "scripted" films and research reports, the Branch is called on continually to edit and provide titles for film footage exposed by research personnel.

The animation production activities of MAPB have developed and moved forward to the extent that sequences of considerable complexity have been photographed. However, it is the policy of the Branch to use animation only in those instances where the action cannot be covered by live photography or where diagramatic presentations enhance the communication effectiveness of a film.

D. Problems

One problem which was reported last year continues to be very bothersome. The water supply to the photographic service area contains dirt and other contaminating substances which cause spots on both negatives and prints. Filters have been installed, but some contaminants can still pass through the filtration equipment. Water coming from the filters is sometimes so low in pressure that the temperature controls do not work properly. Ideally, wash water should be at the same temperature used for the development process, i.e. 68° - 70° F. Wash water of a temperature much higher than this can ruin the pictures.

A most pressing problem is space. MAPB is not centralized, and the physical separation of art services from photographic services, with the motion picture services in another building and the Branch Office separated from all its Sections, makes administration difficult and coordination a formidable task. Added to the management problems that separation of the different Sections creates, there is the dismal location of the motion picture service in a sub-basement area. The model and moulage activity is also located in the sub-basement of Building 1 and it suffers by being detached from the rest of the medical illustration program.

Another problem is the difficulty of recruiting competent photographic laboratory personnel. Working in a photo darkroom has little glamour. At times the work is tedious and repetitive, and the photographic laboratory technician usually has little social contact while he is working. The civil service pay scale for a routine photo lab technician is not sufficient to attract people. MAPB has several good photographic laboratory employees but there is no backstop in case one goes on leave or falls ill.

E. Program Plans

Contracting for outside services is carried on in the areas of drafting, photography, motion pictures, and general illustration. It is believed some of these contracting activities can be centralized and a gain in efficiency realized. During the report period, travel and purchasing have been centralized in the Branch Office, and better budgetary control of both materials and equipment has occurred.

Within the next fiscal year (1967) it is planned to install and have in operation equipment to automate the processing of photographic prints.

MAPB has never had a photo-science specialist as a member of its staff. The quality and variety of the still and motion picture photography requested from MAPB is of such a complex nature that the addition of a photo-scientist to the staff is indicated. To fill this need, a recent graduate of the College of Graphic Arts and Photography, Rochester Institute of Technology, has been recruited and is expected to join the MAPB staff at the beginning of next fiscal year.

The picture and negative files have grown to such an extent that the "home-made" filing system has become overburdened. A better, more flexible system is required. Steps have been taken to learn the features of some other filing systems used by organizations with much larger picture collections. During the next year it is hoped that a new system for filing NIH photos will be adopted. The changeover will require time, equipment, and the addition of temporary personnel.

The NIH exhibits program has never had the benefit of an overall, expert evaluation. During the summer of 1966, the Director, Office of Learning Resources at the University of California School of Medicine in San Diego will be here for the express purpose of initiating a study to determine the effectiveness of our exhibits for conveying ideas.

Steps have been taken to increase the motion picture animation resources. Fortunately, an arrangement was made with Wexler Film Productions in Los Angeles to have one of the MAPB artists receive animation apprentice training for three months. If this training program proves successful, MAPB will probably extend the same opportunity to at least one other MAPB artist.

F. Publications

Bartner, H.C.: Sketching retinal changes. Visual Medicine. Vol. 1, No. 2, June 1966.

Taylor, V.E.: Classification and evaluation of photographic personnel. Industrial Photography. 14: 28, Nov. 1965.

Wilcox, M.A.: Paraplex - A new biological embedding resin. Med. & Biol. Ill. Vol. 15, No. 4, Oct. 1965.

DIVISION OF RESEARCH SERVICES

Summary of Branch Activities July 1, 1965, through June 30, 1966

LABORATORY AIDS BRANCH

Dr. Robert J. Byrne
Branch Chief

I. SUMMARY

The Laboratory Aids Branch is responsible for the production of laboratory rodents and rabbits; for the production of germfree and pathogen-free animals; for the conditioning of dogs, cats, primates, and feral animals; for special animal-related services; for production of animal blood and animal blood products; and for the preparation of bacteriologic and tissue culture media and clean glassware.

During the past year, planning for all phases in the development of the NIH Animal Center continued. Experience gained during the first full year of operation in the new Phase I facilities, and the response of NIH investigators to the resources have been favorable. There has been a marked increase in demand for services in both the Farm Animal Unit and the Dog and Cat Conditioning areas.

Public interest in the NIH policy on the acquisition of dogs and cats for biomedical research has resulted in increased demands on staff time to answer outside inquiries. Answers to inquiries reflected our established policy of improving dog and cat procurement methods, upgrading the quality of dogs and cats for issue, and seeking means of utilizing other animals and resources whenever feasible.

Space limitations have prevented significant increases in production of conventional rodents and rabbits. Approximately 10 percent of total NIH needs for these animals are met through outside purchase.

Pathogen-free animal production and RIF-free egg production have been established on a regular basis and are filling a significant NIH requirement.

Renovation of the media production area and the introduction of night shift (4 p.m. to 12:30 a.m.) into both Media and Glassware Preparation areas have provided greater operating efficiencies and expanded services.

Production of Laboratory Rodents and Rabbits

A. Objectives

To furnish high quality laboratory rodents and rabbits by production in closed colonies under a systematic breeding program.

B. Current Programs

Production Program: During the past year, emphasis has been placed on improving management practices designed to: (1) balance the animal production program to keep surpluses to a minimum; (2) reduce administrative overhead through simplified accounting procedures.

The volume of animals produced remained about the same due to space limitations.

Genetics Program: This program is designed to maintain the genetic integrity and desired features of the inbred lines of mice, rats, and guinea pigs. These characteristics are insured through the systematic use of immunogenetic testing and by maintaining a selective program. Close liaison is maintained with Institute/Division investigators to determine suitability of issued inbred animals.

Planning Program for NIHAC Phase II: This program is concerned with the development of operational plans, equipment, training, and the logistics necessary to the implementation of the Phase II production facility at NIHAC. The professional staff continues to visit other facilities to gather additional information on producing and monitoring pathogen-free animals.

C. Program Progress and Accomplishments

During FY 1966 the Laboratory Aids Branch again produced over one million laboratory rodents and rabbits, for a total dollar volume exceeding $920,000. Many animals were bred to specific investigator requirements. Shifts in the production operation were closely geared throughout the year to research requirements. Approximately 10 percent of NIH intramural needs for rodents and rabbits were purchased directly from outside sources.

In collaboration with the staff in the Pathogen-free Production Unit, the Genetics Unit was able to establish inbred strains of mice in isolators. The DBA/2N strain, characteristically a low producer, was the first strain selected.

A Program of Requirements for Phase II NIHAC was completed with RFPB during the year. Considerable effort was devoted to answering inquiries from the Bureau of the Budget relating to this building program.

Planning activities during the past year have been broadened to provide for an orderly transition of activities from Bethesda to NIHAC over an extended

period of time and have included consideration of total NIH needs for animals and methods of supplementing in-house production. Plans have been formulated for the training of personnel and a systematic rotation of experienced animal caretakers, in order that a competent force will be available to staff the NIH Animal Center.

A Policy and Procedure Memorandum was developed which established better defined guidelines for the handling of surplus animals.

D. Problems

The most significant problems in the Animal Production area during FY 1966 related to diseases associated with breaks in sanitation. For example, salmonellosis necessitated eradication of the Strain 13 guinea pig production colony and the Flemish rabbit colony was almost decimated due to coccidiosis. Fortunately the genetic back-up colony of Strain 13 guinea pigs did not become infected and could be used to establish a new production colony. Overall sanitary practices and the physical environment have been carefully reviewed with the assistance of the Environmental Services Branch and corrective actions have been taken wherever feasible. There are remaining problems, associated with the physical plant, that probably cannot be satisfactorily corrected without considerable financial investment. The ultimate phasing out of animal production in wings B, C, and E of Bldg. 14 precludes any major construction or alterations.

E. Program Plans

The development of plans for transportation and logistical support for the NIH Animal Center will dominate program planning during the year. New equipment is being developed and evaluated, methods of conversion from conventional to pathogen-free of many inbred strains are in the developmental phase, and plans are being made for training personnel for the pathogen-free operation prior to the completion of the facility.

During the coming fiscal year, further concentrated effort will be made to balance our animal production programs to meet the research needs and at the same time to keep the numbers of excess animals to a minimum. A more comprehensive survey of Institute/Division rodent and rabbit needs for FY 1967 is now being completed and should be helpful to program planning and implementation. It is anticipated that APS:LAB will play a more active role in coordinating outside procurement of rodents and rabbits for intramural programs.

There will be an increased effort to improve sanitation, making use of the available resources, yet keeping within the limitations placed on major alterations. Breeding units of the valuable inbred strains of guinea pigs will be housed in modified Horsfall units. Consideration will be given to contract arrangements as a back-up on valuable inbred strains of animals. More effective means of monitoring will be established.

Production of Special Animals

A. Objectives

To furnish NIH investigators with laboratory animals whose associated flora and fauna is defined in some specific manner, i.e., germfree, RIF-free, Lactobacillus monocontaminated, pathogen-free, etc. To develop systems and methods for more efficient production of a greater variety of these animals.

B. Current Programs

Germfree rats, mice, and guinea pigs are being produced in stainless steel and flexible plastic isolators. A careful monitoring program assures that these animals are germfree. Limited attempts to develop a diet and system for raising cesarean-derived germfree hamsters have not been successful.

Pathogen-free rats and mice are provided through the breeding of cesarean-derived animals within a barrier system. Current methods of producing pathogen-free animals are critically appraised for their suitability to future operations at NIHAC. A program to provide pathogen-free guinea pigs is underway.

Fertile hen's eggs free of the Resistance Inducing Factor (RIF) are produced by maintaining a flock of chickens within the barrier system. The birds are housed in individual cages, bred by artificial insemination, and regularly monitored for RIF.

C. Program Progress and Accomplishments

The current rate of issue of germfree animals to NIH laboratories approximates 11,000 per year. This is a 40 percent increase over issues a year ago. This rate also represents the peak potential of present space, personnel, and isolators. There have been increased requests for germfree animals, equipment, and technical information by NIH investigators outside the regularly established germfree laboratories.

Pathogen-free animals are being issued to NIH laboratories at the rate of 26,000 animals per year. This is an increase of approximately 60 percent over a year ago. Recently there has been a large increase in requests for pathogen-free rats. Production is being increased to meet this demand.

The second generation of RIF-free chickens is now in production. Productivity and fertility are good, with the rate of issues being 8,800 eggs annually.

Quality control and microbiological monitoring programs are being utilized for all groups of animals. Contamination rates are low. Evidence of high quality in all animal groups comes not only from the monitoring program but also from increasing requests of NIH investigators for these animals.

D. Problems

Attempts to establish production of germfree hamsters and pathogen-free guinea pigs have been unsuccessful. New approaches will be tried and in the case of pathogen-free guinea pigs there is confidence that success will be achieved.

Maintaining adequate positive air pressure in the pathogen-free production rooms has been a continuing problem. It appears that the basic source of the problem, namely, cracks up to one-half inch wide between the air ducts and the filtered air inlets to the rooms, has been located.

E. Program Plans

Continued efforts will be made to produce pathogen-free guinea pigs and germfree hamsters.

Production of pathogen-free mice and rats will be increased through more concentrated use of the space available.

Plans have been completed to alter the method of production of RIF-free eggs to provide for greater security against contamination. Necessary new construction, renovations, and new equipment have been planned in detail. The new system calls for two flocks to be housed in isolators; one flock to be in Building 14G at Bethesda and the other in a new building at the NIH Animal Center. A prototype isolator is now undergoing design evaluation. When the new flocks are established in the isolators, the present flock of birds will be phased out.

Quarantined and Conditioned Animals

A. Objective

To furnish quarantined and conditioned dogs, cats, primates, and feral animals to NIH investigators.

B. Current Programs

The system of initial and quarterly inspections of dog and cat vendor facilities is designed to maintain established produrement standards. Purchased animals are quarantined, conditioned, vaccinated, or tested for specific diseases, and issued for investigational use. Efforts are made to purchase and issue animals which best suit the needs for specific research programs.

C. Program Progress and Accomplishments

Projected dog issues will be approximately 5,100 as compared with 4,532 for FY 1965. Cat issues will be approximately 2,800 as compared with 2,670 in FY 1965. Primate issues decreased from 3,161 in FY 1965 to a projected 2,900 in FY 1966.

Inspection of vendors' premises has resulted in the cancellation of one contract. Although the physical plant of this vendor was deemed suitable, inspections revealed violation of acceptable practices. The vendors remaining under contract have, with the advice and guidance of our staff, continued to improve their facilities and management. It is anticipated that these vendors will fulfill the requirements of pending federal legislation regulating the interstate shipment of dogs and cats.

A tuberculosis-like disease has been recognized in opossums under quarantine. The causative organism has been isolated but is as yet unidentified. A tuberculin testing program has been instituted and is being evaluated.

Over the past year there has been a significant change in the primate holding activity as a reflection of Division of Biologics Standards and other Institute program changes. Whereas in the past, quarantined monkeys were issued at a predictable rate, there is now a distinct trend toward prolonged holding. DBS now requests that a fixed inventory of rhesus monkeys be maintained at all times to meet contingencies. A number of other Institutes are turning to us for holding of primates on long term experiments.

A program was instituted in which all dogs and cats are now identified on receipt by tattooing a number in the ear.

D. Problems

Procurement of suitable dogs continues to be a problem and is frequently compounded by outside harrassment of vendors by human society groups. The frequency of visits and inquiries connected with missing dogs has been time-consuming, despite the development and implementation of a formal system for handling.

While improved facilities and treatment methods have reduced dog and cat mortality during the quarantine period, diseases are still a costly problem and preclude issuance of ideal research animals.

E. Program Plans

In collaboration with Comparative Pathology Section, a systematic investigation of the viral and bacterial diseases causing high morbidity and mortality rates in both the dog and cat quarantine populations is projected. The roles of stress, housing, and husbandry practices will also be considered.

Procurement specifications for chimpanzees will be modified to help obtain more healthy animals at the time of purchase, and intensive clinical and laboratory care should minimize disease and death.

A. Objective

To characterize and improve the health of laboratory animals used in the medical research programs of NIH.

To study the causes of naturally occurring laboratory animal diseases and to implement methods of prevention, treatment, and control.

B. Current Programs

The problem of disease control in closed colonies of rodents and rabbits is met through a multi-disciplined approach to monitoring. Comparative Pathology Section pathologists and microbiologists work closely with the Animal Production Section to control and eliminate infectious diseases.

The Section also provides laboratory diagnostic service in conjunction with the disease control programs of the Dog, Cat, and Primate Quarantine Units and the Farm Animal Unit, and provides bacteriologic monitoring service for the Pathogen-free Unit.

Control of infectious diseases of dogs and cats is carried out through a program of routine immunization and treatment. Routine control of infectious diseases of primates is approached through a program of tuberculin testing, bacteriologic monitoring, and treatment. The health of farm animals is maintained through a program of disease prevention and by routine testing.

In addition to programs for controlling infectious diseases, there are studies conducted on nutritional and metabolic diseases.

To support health surveillance of colony animals, there is a program to study, under controlled conditions, naturally occurring diseases of laboratory animals.

C. Program Progress and Accomplishments

In September 1965, salmonellosis occurred in the closed animal colonies for the first time in more than 10 years. Early detection and diagnosis made it possible to confine the disease to one room of guinea pigs and thereby avoid a major health disaster in the colonies. All of the laboratory animal species are susceptible to salmonellosis. The affected guinea pigs, comprising the Strain 13 production colony, were quickly moved from the NIH closed colonies to isolation quarters at NIHAC. Efforts to save these animals were abandoned when it was shown that many of the animals developed subclinical infections. They were disposed of in large scale terminal experiments conducted by NIH investigators who have been the main users of these animals. The production colony is now being restocked from the foundation genetic colony, which was shown by extensive testing to be free of infection. The isolates from a number of infected animals in the production colony were each shown to be Salmonella typhimurium var. copenhagen.

The results of transmission studies conducted in Horsfall cages demonstrated that other colony animals, especially mice, were highly susceptible to the agent by both oral administration and contact exposure.

An explosive outbreak of coccidiosis occurred in the rabbit colony in October 1965. Three rooms were involved. Using the "test and kill" method in combination with furacin therapy, the infection was eliminated within three weeks after the first oocysts were found. Nearly 100 percent of the animals were lost from one of the rooms, only 10 percent from the other two rooms. Fecal examinations for oocysts had been negative for 25 months prior to the 1965 outbreak, using a composite sampling method which tests 40 percent of the rabbits in each room per month. In later studies of the outbreak, it was found that the severely affected rooms was located adjacent to a building entrance which opened onto the platform of the cage washing building. Soiled cages from a rabbit colony not under LAB supervision had often been stored on the platform. This colony was examined and found to be heavily infected. The infection in the colony has been eliminated and corrective preventive measures have been taken.

For many years, approximately 70 percent of the newly procured dogs of the quarantine colony have suffered from the effects of a respiratory syndrome variously described as kennel cough, bronchitis, tracheobronchitis, distemper, etc. At present, the disease is conservatively estimated to cost NIH $40,000 a year. Studies conducted during the past year have suggested that the syndrome is a bacterial disease precipitated by stress (transportation, new quarters, change of feed, vaccinations, etc.). On the basis of bacteriology, serology, and vaccine studies, Bordetella bronchiseptica is suspected as playing a major etiologic role. Accordingly, sulfonamides are being administered to all dogs during their first ten days in quarantine and efforts are being made to reduce stress.

Attempts to produce chronic murine pneumonia in germfree rats by exposing them to Hemophilus sp. and a common diptheroid of rats, have been unsuccessful to date. Similar studies are in progress using Bordetella bronchiseptica. Since the results of previous vaccine studies suggested that chronic respiratory disease (CRD) lesions may result from hypersensitivity to Bordetella bronchiseptica, both vaccinated and non-vaccinated rats are being used. Serum electrophoresis studies have shown that rats with CRD have unusually high globulin levels. It appears that serum globulin values may be useful in diagnosing CRD in live rats. Heretofore, diagnosis has depended entirely upon the demonstration of typical lung lesions at autopsy.

A serious neurological disease of cats in the quarantine colony evidently was correctly diagnosed as thiaminase toxicity. The affected animals responded to treatment with thiamine and the condition disappeared from the colony when the principal diet was changed to one having less fish content.

The use of other than domestic animals by NIH investigators has continued to create disease control problems. A tuberculoid disease occurring sporadically in the opossums has not been identified, and attempts to develop a workable tuberculin test procedure have been unsuccessful. Recently a pleomorphic acid-fast bacillus cultured from one of the opossums was found to

produce lesions in rabbits similar to those found in the opossum. The results of a large battery of tests on the bacillus thus far indicate that it is a mycobacterial species exhibiting characteristics of both the mammalian and the atypical group.

D. Problems

The Comparative Pathology Section still has not been successful in filling a vacancy for a veterinary pathologist. Obvious salary deficiencies constitute a major problem in this regard. During the year, the Section also lost its clinical pathologist, a man with over 10 years' experience in laboratory animal medicine. This man left for an administrative position.

E. Program Plans

The ongoing programs outlined above will continue.

Research and Development (Collaborative Program)

A. Objectives

To conduct, in collaboration with Research Facilities Planning Branch, a research and devlopment program designed for improving animal facilities, caging, and automated feeding, watering, and sanitary systems.

B. Current Programs

Developmental projects are underway which will produce cage systems designs to be implemented in Phases 1A and II of NIHAC and to support the developmental program in the Pathogen-free Unit.

Materials testing projects are underway which will determine the most suitable animal care equipment comercially available for LAB and the Institutes. Part of this program is in collaboration with Supply Management Branch.

C. Program Progress and Accomplishments

FY 1966 was devoted primarily to the development of caging systems for Phases 1A and II of the NIH Animal Center.

Late in the year, the primate cage was redesigned to provide a more efficient system and provide better housing for the primates. Prototypes were designed and constructed and evaluation should be complete early in FY 1967 for use by the A/E.

The study of photoperiodism in mice was completed during FY 1966. The results indicated that four hours of fluorescent lighting daily produced greater numbers of young born, but that the higher mortality reduced the yield at weaning to the same as 12 hours of lighting. Twelve hours of controlled lighting are recommended for mouse breeding colonies.

During the experimental phase of the above study, it was observed that severe alopecia occurred in all of the animal populations. A light source study was started in October 1965 to compare fluorescent versus incandescent lighting with regard to this problem. Results are not yet available.

The original concept of a water flushing system in conjunction with pans beneath rodent cages has proven impractical. An eight-month evaluation period revealed that rats did not breed well and that respiratory diseases were encountered with alarming frequency. A second concept is being evaluated which involves a conveyor belt in place of cage pans, with a mechanical scraper to remove the debris from the moving belt.

During the year a pre-wash unit was developed and installed on a tunnel washer in Building 14F-1. This was the result of constant problems with clogging of the screens and pumps with sawdust and debris which the vacuum system did not completely remove. The new unit provides a combined vacuum and cleaning system and pre-rinse which flushes directly to the sewer in one operation prior to entry into the cagewashing machine itself. This unit has proved very successful in solving the problem.

D. Problems

The amount of time the members of the R&D Team have been able to devote to their mission has been very limited this year. The RFPB member has been involved in other projects in his branch, and the LAB member has been confronted with additional administrative responsibilities as Chief of APS and in NIHAC planning. If this program is to continue as scheduled, additional personnel should be considered, or more of the experimental work must be contracted. The latter is not desirable since evaluations should be made in LAB colonies and coordinated with LAB programs.

E. Program Plans

During the coming year a study will be made to determine the effect of various light intensities on the productivity of mice.

A prototype rabbit facility will be developed at NIHAC to study the production of healthier animals at a significantly lower cost than current methods. It is planned that an existent structure will be slightly modified to permit evaluation of a more economical production method in a climate of this geographical area.

A prototype automatic rat and mouse feeder for pathogen-free operation will be acquired and evaluated.

Several systems will be designed for study with regard to the transportation of pathogen-free animals from NIHAC to the Bethesda campus.

During the year, a proposal was received for a new type of drinking valve that should eliminate many of the problems associated with the present automatic water system which is under evaluation. Prototypes of this system have been ordered and will be evaluated during the next fiscal year.

Special and Consultive Services to the Institutes

A. Objectives

To furnish special services pertaining to animal programs and animal facilities as requested by the Institutes/Divisions. These services include consultation on animal problems, furnishing of surgical and X-ray services for dogs, cats, primates, and farm animals, and the production and issue of blood and blood products. The experimental surgery staff has furnished consultative services to investigators on such subjects as anesthesia induction, anatomy, and physiology.

B. Current Programs

LAB veterinarians and other specialists furnish NIH investigators with a variety of information on normal animal anatomy and physiology, proper husbandry practices, and disease problems.

Outside inquiries on animal programs and facilities are answered and material for congressional correspondence prepared.

Animal X-ray and surgical services are provided in Building 28. These programs are supported by an intensive care team and long-term holding facilities. Surgical services and post-surgical holding for ungulate animals are provided in the facilities at NIHAC.

LAB staff members are active in the design of animal facilities to be utilized for their programs, such as the NIH Animal Center, and are consulted by the Institutes on design of animal facilities.

LAB provides the space and caretaking service for a limited number of radioactive isotope experiments.

LAB maintains a pilot dog and cat breeding facility in Building T-8 at NIHAC. The prototype white hounds, developed in the dog breeding program, are being utilized to study the feasibility of developing a canine blood donor bank to service experimental surgery in the National Heart Institute.

C. Program Progress and Accomplishments

Outside Inquiries: During the past year, there has been an increased public interest in the problems associated with the procurement of dogs and cats for biomedical research. As a result, we have had a marked increase in public and congressional inquiries concerning both NIH and national programs using animals in biomedical research. At the same time, we have been called on with greater frequency by the outside community for our council on dog and cat procurement specifications, our experiences in NIHAC facilities, and the dog and cat breeding program. While time consuming, these inquiries and

contacts have given our staff new insights into the problems and have furnished an opportunity to contribute to their solutions.

Continued efforts to improve methods for procuring, conditioning, and utilizing dogs and cats for biomedical research have led to a number of significant developments:

a. Utilization of calves and other ungulates as replacements for dogs in surgical studies.

b. An exploratory program to determine the feasibility of a canine blood donor system to fill the National Heart Institute needs for blood in experimental surgery. If successful, this would reduce the annual need for random source dogs by 2,000 and, at the same time, furnish a far better quality of blood.

c. Contract specifications have been developed for commercial breeders to provide a satisfactory supply of dogs specifically bred for research purposes. When implemented these specifications will insure close NIH control over program to insure the lineage of the animals, their immunization status, and their health throughout rearing.

Experimental Surgery: Although requests for X-ray services continue at the same rate, those for assistance with radioisotope studies seem to have increased. Space, special housing, and care for radioactive animals have been provided as needed. Surgical space has also been provided for a number of tracer studies. Facilities for these services remain inadequate.

The number of surgical cases per month has increased from approximately 40 in FY 1965 to approximately 50 at present. Procedures, in some instances, have been of a more ambitious nature than in the past. While animal surgical facilities at NINDB are undergoing renovation, LAB has provided operating room space and equipment to that group for their work with primates.

The number of physiological sampling procedures performed monthly has doubled. For some studies, we have begun to perform cross matching of blood prior to transfusion.

The long-term holding area has housed about 30 more dogs per month than in FY 1965. Not as many farm-type animals have been held in the Surgery Unit as in FY 1965.

Farm Animal Activity: There was a substantial increase in requests for post-operative care of animals, particularly calves and sheep. The calves were mainly heart surgery subjects. The increased use of this species for open heart surgery has necessitated the keeping of increased numbers of bovine blood donors and has also increased the labor required for the drawing and handling of blood.

Carnivore Activity and Blood Program: The dog and cat breeding activity formerly housed in outdoor facilities T-3 and T-4 was moved into Building

T-8. Blood grouping studies were conducted on the hounds and mating systems between A-negative animals were established in anticipation of conducting blood donor trials.

D. Problems

We have lacked adequate facilities to conduct radioisotope experiments on animals.

There have been difficulties involved in hiring technical people for the blood donor program at Poolesville, but this problem may be resolved soon.

There has been a very heavy administrative load placed on the Branch and its sections due to outside inquiries on animal procurement and production problems. However, a more efficient means of handling such inquiries is being developed.

E. Program Plans

Modernization of equipment and surgical instrumentation is planned for the experimental surgery area in Building 28. Closer controls on use of the facility should derive maximum use of the resources available.

We anticipate the realization of the canine blood program and outside procurement of specially bred dogs and cats during the next fiscal year.

There will be increased emphasis on technical, in contrast to caretaker, support in the Farm Animal Unit.

It is anticipated that the Branch will become increasingly involved, as requested, in NIH Institute/Division animal programs.

There will be increased Laboratory Aids Branch involvement in the outside procurement of rodents and rabbits for NIH intramural programs.

Media Production

A. Objective

To produce and issue high quality bacteriological and tissue culture media. To furnish consultative services to the Institutes on related problems.

B. Current Programs

Media production is geared to meeting Institute needs as to volume and type of media. To fulfill its mission, the Media Unit employs automated equipment wherever possible and makes use of an efficient cross-reference system of record keeping.

The quality control program of media production was expanded this year to include efficiency testing of a greater number of tissue culture media

concentrates. The Unit has continued to provide concurrent testing of its media in several established tissue culture cell lines. In addition, an improved method of sterility testing has been incorporated into the production procedures.

The demand for tissue culture cells in excess of those required in quality control has continued. No concerted effort, however, has been made to increase the supply of these cells.

A limited program to provide storage of representative cell lines from various investigators in a frozen cell bank was initiated.

The Media Unit assists in the development of improved methods for procuring and storing animal blood, serum, tissues, etc. The Unit also processes these materials for issue.

C. Program Progress and Accomplishments

Based on the first nine months, the number of requisitions processed in FY 1966 will again total approximately 12,000. This is the third year with a monthly average of 1,000 requisitions processed.

In terms of volume of media produced, the projected figures indicated 43,000 liters of bacteriological media and 39,000 liters of tissue culture, an increase of 10 percent.

The Media Unit contracted with a commercial source for the supply of sheep and rabbit blood agar plates in disposable plastic petri dishes. A projected nine-month total of 49,000 commercial plates will be issued in FY 1966 in addition to 193,000 plates of various other types poured by the Media Unit. When compared to a total of 170,000 plates issued in FY 1965, this is a significant increase in the demand for this service.

A projected total of 68,000 tubes of tissue culture cells were issued in FY 1966. All investigators using these cells have continued to report satisfaction with the cells received.

The intensive renovations mentioned in last year's report and recommended in the DRS sponsored space utilization study were completed in April, 1966. Several new concepts in clean room design were incorporated into the renovations, and preliminary results indicate the Unit should have an increased capacity for producing sterile media

The night shift initiated in mid-February is not yet fully staffed. Even with the shift operating at half-strength, there has been improvement in ability to take care of urgent requests.

At the request of several investigators, the Media Unit has undertaken a limited program of maintaining samples of frozen cell lines of various types. The investigator supplies a sample of the desired cell line which

is passed several times to increase the volume of cells. These cells are then frozen and stored in liquid nitrogen to be returned to the investigator when required.

The Media Unit has expanded the use of commercial powdered tissue culture media. Use of this type of product for bulk orders has allowed the Unit to devote more time to the preparation of unusual types of media not commercially available.

D. Problems

The lack of adequate numbers of delivery containers continues to be a problem. Without a steady flow of these containers back to the Media Unit, it is impossible for the Unit to adequately meet the demands of the investigators.

In recent months increased difficulty has been experienced with transporting prepared media to other buildings. On several occasions entire batches of media have been received in the investigators laboratory in an unusable condition. There would be definite advantages to a system of transportation with responsibility for direct delivery to the investigator's laboratory under Branch or Section control.

E. Program Plans

Ongoing programs described above will be continued.

Efforts to further improve methods of automating the production of bacteriological and tissue culture media will be made. Several items of automation equipment are under consideration.

With the increase in production areas for sterile products, a system of monitoring the contamination level in these areas is needed. The monitoring will be done by personnel in the Media Unit so that problems can be corrected immediately.

Glassware Preparation

A. Objectives

To provide an adequate supply of clean laboratory glassware for research programs.

To furnish consultative services on problems associated with laboratory glassware.

To furnish cagewashing service to the Clinical Center, Auburn Building, and Patuxent River Laboratory.

To furnish ethylene oxide gas sterilization of heat labile patient and laboratory equipment.

B. Current Programs

In addition to filling Institute needs for glassware, the Unit develops management and technical methods for quality control and for improving the distribution and return of glassware.

The cagewashing unit is now washing the rubber backed carpets for the Clinical Center and outlying buildings.

C. Program Progress and Accomplishments

Glassware issues projected through the end of the fiscal year will total approximately 7,683,000 pieces, an increase of 9.1 percent over FY 1965.

Additional funds became available to the Unit for the purchase of new glassware during the last half of the fiscal year. It is hoped that this will enable the Unit to establish more adequate reserve stocks of sterile glassware, particularly serological pipettes.

A night shift was inaugurated in the Glassware Unit in February. Even with a partial crew on duty, virtually all overtime used for processing glassware has been eliminated.

A new deionizer was installed this year increasing the Unit's capacity to produce deionized water by 100 percent. It is quite evident that there would not have been enough deionized water available for use by the night shift without this unit.

A "Cryotherm" gas sterilizer was installed this year so that small items such as telephones, plasticware, clothing, etc., can be sterilized more quickly and efficiently. By using this sterilizer we can add controlled heat and humidity to the chamber, thereby speeding up the sterilization process.

A projected total of over 369,000 cages, racks, and associated pieces of equipment will be processed this year. The cage washing unit began serving the DBS facilities in Building 14.

D. Problems

The shortage of containers required to handle glassware continues to be the most serious problem. Metal trays for return of dirty glassware are in critical shortage.

E. Program Plans

Efforts to automate pipette wrapping and other operations will continue. A new type of plastic sheeting that will withstand dry air sterilization and yet is adaptable for use on a wrapping or sealing machine has been located.

Testing of "Corex" pipettes will continue. The manufacturer claims they will last six times longer than regular pipettes.

In cooperation with the Supply Management Branch and several plastic manufacturers, effort is being made to obtain a plastic container to replace the expensive stainless steel tray now used to transport dirty glassware.

PUBLICATIONS AND PATENTS

Publications

Formal, S.B., Labrec, C.H., Palmer, A.E., and Falkow, S.: Protection of monkeys against experimental shigellosis with attenuated vaccines. J. Bact. 90: 63-68, Jul. 1965.

Gaddis, E.M., Fisher, L.B., Miller, C.E., and Rosenberg, L.E.: Pathologic aminoaciduria in experimental ascorbic acid deficiency. Proc. Soc. Exp. Biol. Med. 120: 185-187, 1965.

Ganaway, J.R., Allen, A.M., and McPherson, C.W.: Prevention of acute Bordetella bronchiseptica pneumonia in a guinea pig colony. Lab. Anim. Care 15: 156-162, Apr. 1965.

Potkay, S., Ganaway, J.R., Rogers, N.G., and Kinard, R.: An epizootic of measles in a colony of rhesus monkeys (macaca mulatta). Amer. J. Vet. Res. 27: 331-334, Jan. 1966.

Films

Pathogen Free Animals. A 15-minute, 16mm, sound film intended primarily to inform visitors to Laboratory Aids Branch about methods used to produce pathogen-free animals, and as an aid in employee training. Produced by LAB and MAPB.

Presentations

Bohner, H.J.: Some practical aspects of producing and distributing germfree and pathogen-free animals. Pharmaceutical Manufacturers Association, Washington, D.C., March 1966.

Byrne, R.J.: Procurement and conditioning of dogs for medical research by the National Institutes of Health. Eastern Shore Veterinary Association, Easton, Maryland, September 1965.

Ganaway, J.R.: Respiratory Disease in Dogs. Ibid.

LeMunyan, C.D.: Photoperiodism in mice. 16th Annual Animal Care Panel, Philadelphia, Pennsylvania, November 1965.

Serial No. DRS-LAB-1
1. Laboratory Aids Branch
2. Animal Production Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: THE EFFECT OF LIGHT ON THE PRODUCTIVITY OF MICE

Previous Serial Number: 4.1

Principal Investigator: Cobert D. LeMunyan

Other Investigators: William R. Clark, Jr., RFPB

Cooperating Unit: Research Facilities and Planning Branch

Man Years (computed for the 12 month period)
Total: 1.2
Professional: .2
Other: 1.0

Project Description:

Objectives: To study the effect of various day lengths on the productivity of mice. To study the effect of various light sources.

Methods Employed: The cycle is varied and the light intensity is held constant with a fluorescent source. To study the effect of light intensity, temperature, relative humidity, and duration of light exposure held constant and the source varied from incandescent to a number of types of fluorescent light.

Major Findings: A definite trend of high birthrate during short light periods to low birthrate under continuous lighting was established. However, due to higher mortality in the short day groups, the yield of weaned animals from the shortest day did not differ significantly from the twelve hour day groups. It was concluded that a controlled daily lighting period of 12 hours is optimum for production of mice.

Data on the second part of the study on the effect of different light sources are still being compiled.

Significance: The findings of this research will be utilized in the planning of the rodent and rabbit buildings at the NIH Animal Center, Poolesville, Maryland.

Proposed Course of Project: The first part of this study is complete. Light intensities and sources are currently under study.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-2
1. Laboratory Aids Branch
2. Comparative Pathology Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: CHRONIC MURINE PNEUMONIA OF THE ALBINO RAT

Previous Serial Number: 4.9

Principal Investigator: James R.Ganaway

Other Investigators: Anton M. Allen

Cooperating Unit: Animal Production Section, LAB

Man Years (computed for the 12 month period)
Total 1.7
Professional: .2
Other: 1.5

Project Description:

Objectives: To isolate and characterize etiologic agents. To perform transmission studies with each suspected agent. To recommend measures for prevention and control of this disease complex under colony conditions.

Methods Employed: Microbiology, immunology, and pathology methods were employed.

Major Findings: Long term transmission studies were performed, using two bacterial suspects (based on isolation studies, viz. diptheroids and a Hemophilus sp.) and limited numbers of germfree rats held in plastic isolators. Neither of these organisms, alone or in combination with each other, appear to be capable of causing chronic respiratory disease in albino rats. A vaccine trial in the General Purpose Rat Colony using Bordetella bronchiseptica antigens, instead of being protective as was anticipated, resulted in accentuated morbidity and mortality. Because of the possibility that hypersensitivity may play a major role in this chronic disease, as indicated by the vaccine trial, the vaccine experiment will be repeated in conventional and cesarean-derived rats held in plastic isolators.

Significance: This disease complex is the greatest cause of morbidity and eventual mortality in rat colonies. Affected rats are unfit for research, particularly when destined for long term or toxicity studies.

Proposed Course of Project: Continuation.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-3
1. Laboratory Aids Branch
2. Comparative Pathology Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: FOCAL NECROSIS OF THE LIVER OF RABBITS

Previous Serial Number: 4.12

Principal Investigator: James R. Ganaway

Other Investigators: Thomas D. Moore
Anton M. Allen

Cooperating Units: Animal Production Section, LAB

Man Years (computed for the 12 month period)

Total:	2.0
Professional:	.5
Other:	1.5

Project Description:

Objectives: To characterize and identify the etiologic agent. To study the pathogenesis of the disease through experimental transmission studies. To recommend measures for control or prevention in the rabbit colonies.

Major Findings: Transmission of the disease from sick to healthy rabbits by contact exposure has continued to be successful. Serial propagation of the etiologic agent in embryonated hen's eggs has been accomplished. The disease has been reproduced experimentally in rabbits by feeding infected egg tissue suspensions. Extreme liability of the vegetative phase and the formation of a spore has been confirmed. Repeated attempts to grow this obligate intracellular parasite in primary and cell line tissue cultures have failed.

Significance: Essential to our knowledge of laboratory animal diseases. This disease continues to exist endemically in the NIH rabbit colony.

Proposed Course of Project: Continuation.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-4
1. Laboratory Aids Branch
2. Comparative Pathology Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: SALMONELLOSIS IN GUINEA PIGS

Previous Serial Number: None

Principal Investigator: Thomas D. Moore

Other Investigators: James R. Ganaway
Anton M. Allen

Cooperating Units: None

Man Years (computed for the 12 month period)
Total: 2.6
Professional: .6
Other: 2.0

Project Description:

Objectives: The study of a salmonella outbreak in a highly inbred guinea pig colony.

Methods Employed: Gross and histopathological techniques, bacteriological, serological, and transmission studies were employed in this study.

Major Findings: One of a pair of Strain 13 guinea pigs from the Animal Production Section submitted for autopsy September 1965, showed marked splenomegaly with focal necrosis of the liver and spleen. The second animal showed only the signs of metastatic calcification. Samples of heart blood from both animals were negative for bacteria. A guinea pig with the above spleen and liver involvement was received the next day. A gram-negative, non-lactose fermenting, motile rod was isolated in pure culture from the spleen. Preliminary biochemical characterizations strongly indicated salmonella. Serological tests produced a positive reaction with Group B antiserum. More extensive biochemical tests from different animals were sent to the Communicable Disease Center, Atlanta, Ga., for species identification. All of the cultures were identified as S. typhimurium var. copenhagen. All other isolations made during this investigation from animals presenting a similar pathologic picture, reacted with Group B antiserum. Two cultures sent to CDC near the end of the outbreak were of the same species and variety.

A fecal survey of the caretakers produced one carrier of salmonella. This isolate was identified as S. heidelberg. A check of fecal samples from 119 guinea pigs of the foundation genetics colony of Strain 13 were negative for salmonella and shigella.

The literature has a paucity of information of salmonella infections in the guinea pig although the probable first observation of salmonellosis in this animal was made by Theobald Smith at the turn of the century. Smith termed the disease pseudo-tuberculosis because of the striking tubercle-like nodules in the livers and spleens of his animals. He later recommended calling this agent Bacillus pestis-caviae and cites the loss of an entire guinea pig stock at Rockefeller Institute due to this agent.

In this outbreak all cagemates of animals diagnosed positive for salmonella by post mortem examination were autopsied. As a result 146 animals were examined, 91 females and 55 males. Of the 91 females, 28 were positive, 63 were negative; there were 13 positive and 42 negative males.

Significance: The unquestioned pathogenecity of this genus for all mammals points out the continuous need for extreme diligence and care in the husbandry of laboratory animals. The general lack of efficacy of antibiotics in completely eliminating this infection and the ease with which the carrier state is induced by this organism, reemphasizes the necessity for careful management.

Proposed Course of Project: Continuation. A report is being prepared for future publication.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-5
1. Laboratory Aids Branch
2. Office of the Chief
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: A STUDY TO DETERMINE THE EPIZOOTIOLOGY OF TYZZER'S DISEASE IN RABBITS

Previous Serial Number: 4.13

Principal Investigator: Charles W. McPherson

Other Investigators: Thomas Moore

Cooperating Units: Comparative Pathology Section, LAB
Animal Production Section, LAB

Man Years (computed for the 12 month period)
Total: Less than .05

Project Description:

Objective: To provide a description of the epizootiology of Tyzzer's disease in rabbits.

Methods Employed: Standard epidemiologic methods of recording and analyzing on cases and controls, such factors as age, sex, ancestry, littermates, previous illness history, parity, and litter size; determination of case incidence according to time, place, and environmental changes.

Major Findings: Tyzzer's disease in rabbits tends to concentrate in certain litters and epizootics are more likely to occur when the population of susceptible age pups is high.

Significance: A certain critical population level of susceptible pups is necessary for Tyzzer's disease to spread epizootically from litter to litter.

Proposed Course of Project: Complete except for writing of final report.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-6

1. Laboratory Aids Branch
2. Office of the Chief
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: THE EFFECT OF CERTAIN BACTERIAL SPECIES ON CHRONIC RESPIRATORY DISEASE OF RATS

Previous Serial Number: None

Principal Investigator: Charles McPherson

Other Investigators: None

Cooperating Units: Comparative Pathology Section, LAB

Man Years (computed for the 12 month period)
Total: Less than .05

Project Description:

Objective: To determine if any of the bacteria used to purposefully contaminate the pathogen-free isolators have any effect on the incidence or severity of chronic respiratory disease in rats.

Methods Employeed: Contaminate isolators with each of the four bacterial species usually used to infect the pathogen-free isolators. Several isolators remain as germfree controls. All rats are kept in a production status for one year. Then they are necropsied and examined for the presence of chronic respiratory disease.

Major Findings: The study is not complete. No data will be gathered until the end of the study.

Proposed Course of Project: Continuation.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-7
1. Laboratory Aids Branch
2. Animal Hospital Section
3. NIH Animal Center

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: INVESTIGATIONS OF EQUINE INFLUENZA

Previous Serial Number: None

Principal Investigator: Julius A. Kasel, LCI, NIAID

Other Investigators: Robert H. Alford, LCI, NIAID
Thomas P. Cameron
Robert J. Byrne

Cooperating Units: Laboratory Aids Branch, DRS
Laboratory of Clinical Investigations, NIAID

Man Years (computed for the 12 month period)
Total: 1.1
Professional: .6
Other: .5

Project Description:

Objective: To determine the clinical and laboratory response, in man and horses, to serially passaged equine influenza virus.

Methods Employed: Equine influenza virus is being passed through 25 ponies grouped into six units. The effects of the virus are observed by clinical responses, virus isolation, and antibody titer increase. Chincogeague ponies are used since they are generally free of naturally occurring influenza antibody.

Major Findings: Viral isolation and antibody titer increase have been consistent in exposed ponies. Clinical response indicates a gradual rise in pathogenicity in subsequent passages. The procedure of nasal washing using a soft flexible rubber catheter increased the chances of virus recovery.

Significance: This study has produced the first detailed description of experimental A/Eq_2 equine influenza under experimentally controlled conditions.

Proposed Course of Project: Studies will be completed by June 1966. A manuscript will be submitted for publication.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-8

1. Laboratory Aids Branch
2. Animal Hospital Section and Comparative Pathology Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: MYCOBACTERIOSIS IN THE OPOSSUM

Previous Serial Number: None

Principal Investigators: Thomas D. Moore
Calvin E. Sevy

Other Investigators: Anton M. Allen
Thomas P. Cameron

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	1.5
Professional:	.5
Other:	1.0

Project Description:

Objective: The identification and delineation of atypical Mycobacterium species isolated from an apparent natural infection.

To study naturally occurring diseases in the opossum caused by Mycobacterium and to develop an effective test and control system.

Methods Employed: Histopathologic and bacteriologic techniques are employed with transmission studies on the suspected causative agent.

Animals dying from naturally occurring diseases are subjected to post mortem examination and culture. Healthy opossums are sensitized with various strains of Mycobacterium tuberculosis and tuberculin tested after an appropriate period of time.

Major Findings: In mid January 1966, a mesenteric lymph node and three tubes of broth each containing a small piece of lymph tissue from an opossum was submitted by the quarantine colony. Post mortem examination had indicated a tubercular-like infection. A suspension prepared from the mesenteric node and another from the pooled tissues in broth revealed acid-fast bacilli in

the latter. Each suspension was concentrated and inoculated into suitable culture media and into guinea pigs. A Proteus sp. and a Streptococcus sp. were isolated before concentration. Within 16 days growth was obtained from the tissue submitted in broth. The mesenteric node has remained negative. The growth consisted of pleomorphic, acid-fast coccobacilli. This organism has been transferred through several passages and has been shown to grow at room termperature and at 42° C. The optimum temperature for growth is, however, 37° C.

Tuberculin tests with avian and mammalian antigens on guinea pigs inoculated with both tissue suspensions were negative at 6 and 11 weeks. No gross pathology was evident in these animals autopsied at 6 weeks. At 11 weeks all animals showed splenomegaly and liver nodules.

Animal inoculation studies to date with the isolated organism reveal definite pathogenicity for the rabbit. Splenomegaly and liver nodules are the most striking features of the infection. Tuberculin tests on rabbits and guinea pigs inoculated with the organism are negative at 3 weeks although patent infection is also occurring in the latter animals.

The work on this agent thus far indicates a mycobacterial species exhibiting characteristics of both the mammalian and the atypical group.

Significance: The documentation of mycobacterial infections in the opossum in his natural habitat is apparently nonexistent. The continued use of this animal for investigative purposes necessitates clarification of this problem.

This feral animal is becoming increasingly used as a research animal, but little is known of its specific diseases. Two cases with typical tubercular lesions have been found by the NIH Quarantine Unit and have stimulated research in this particular area. The most serious deficiency in the surveillance of the opossum for tuberculosis is the lack of defined testing methods. In order to protect investigators using this animal it is felt that a reliable means must be determined.

Proposed Course of Project: Continuation:

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-9
1. Laboratory Aids Branch
2. Animal Hospital Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: EVALUATION OF ATTENUATED SHIGELLA VACCINES

Previous Serial Number: 4.5

Principal Investigator: Samuel B. Formal, WRAIR

Other Investigators: Howard C. May, Jr.

Cooperating Units: Laboratory of Immunology, Walter Reed Army Institute of Research

Man Years (computed for the 12 month period)

Total:	3.15	(This is a fulltime project for Dr. Formal
Professional:	1.10	and his 2-man staff.)
Other:	2.05	

Project Description:

Objective: To attempt to develop and evaluate attenuated, non-pathogenic forms of Shigella organisms for potential use as an immunogenic agent to protect rhesus monkeys against these organisms while under quarantine.

Methods Employed: Hybrid organisms produced by mating Shigella organisms (of several species and serotypes) with E. coli will be given via the oral route by stomach tube. Animals will be challenged with fully virulent organisms at varying intervals to determine susceptibility to the virulent agents. In later studies, 50% of the newly arriving rhesus monkeys will be treated, followed by close observation for differences in incidence of shigellosis between treated animals and untreated controls.

Major Findings: One dose of the hybrid organisms given orally affords protection to challenges with virulent organisms of the same serotype.

Significance: About 15% of the rhesus monkeys imported into the Primate Quarantine Unit shed Shigella organisms of several species and serotypes. The clinical syndrome accredited to Shigella spp. is an acute, bloody

diarrhea which costs more in treatment, loss of condition, and animal deaths than any other disease encountered in primates with the Primate Quarantine Unit colony. An immunizing agent of this nature would greatly decrease problems in conditioning primates and increase the quality of animals issued by the Primate Quarantine Unit.

Proposed Course of Project: Continuation of studies to evaluate the value of such vaccines in the quarantine of primates. A manuscript has been submitted for publication.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-10
1. Laboratory Aids Branch
2. Animal Hospital Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: AN EPIZOOTIC OF MEASLES IN A COLONY OF RHESUS MONKEYS (M. MULATTA)

Previous Serial Number: 4.6

Principal Investigator: Stephen Potkay

Other Investigator: James R. Ganaway
Roy Kinard
Nancy G. Rogers, DBS

Cooperating Units: Comparative Pathology Section, LAB
Laboratory of Viral Immunology, DBS

Man Years (computed for the 12 month period)
Total: Less than .05

Project Description:

Objective: To determine the etiology of a condition in the rhesus monkeys of the Primate Quarantine Unit, which interfered with T.B. test readings.

Major Findings: Serology showed that the disease was measles (rubella) and clinical observations were those of conjunctivitis, maculopapular skin rash, and varying degrees of facial edema and erythema. The clinical features of the natural occurrence of this disease have not been reported previously.

Significance: Clinical diagnosis of measles can now be made in the primate quarantine colony. Interference with T.B. test readings by measles infection may be avoided by postponing such tests.

Proposed Course of Project: Completed.

Honors and Awards: None

Publications: Potkay, S., Ganaway, J.R., Rogers, N.G., and Kinard, R.: An epizootic of measles in a colony of rhesus monkeys (macaca mulatta). Amer. J. Vet. Res. 27: 331-334, Jan. 1966.

Serial No. DRS-LAB-11
1. Laboratory Aids Branch
2. Comparative Pathology Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: FEBRILE HEMORRHAGIC DISEASE OF MONKEYS

Previous Serial Number: 4.8 see also 4.7

Principal Investigator: Anton M. Allen

Other Investigators: James R. Ganaway
Nicola Tauraso, DBS

Cooperating Units: Laboratory of Virology and Rickettsiology, DBS

Man Years (computed for the 12 month period)

Total:	2.5
Professional:	.5
Other:	2.0

Project Description:

Objective: To study the pathogenesis of the disease.

Methods Employed: Pathology and microbiology methods were employed.

Major Findings: The clinical features and lesions of this highly fatal disease of monkeys were described in the 1965 Annual Report. Continued histopathology studies have borne out the original findings of hemorrhage in many tissues, systemic vascular stasis, glomerulo-nephrosis, and widespread necrosis of lymphatic tissues. Similar lesions are being found in monkeys given the disease experimentally (transmission studies now being accomplished in closed quarters by the Laboratory of Virology and Rickettsiology, DBS). All evidence to date indicates that the disease is of viral etiology and is not Kyasanur Forest disease. The disease has not occurred in man and has not been successfully transmitted to animals other than the monkey.

Significance: The disease has not been reported in the literature. The causative agent has not been identified and therefore the health hazard to man is unknown. The disease may be introduced into the NIH primate colony again in the future.

Proposed Course of Project: Continuation of pathology studies in conjunction with transmission and virus isolation studies by DBS.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-12
1. Laboratory Aids Branch
2. Animal Hospital Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: EFFECTS OF CERVICAL VAGOTOMY ON WATER AND ELECTROLYTE SECRETION IN THE CHRONIC CARDIAC DENERVATED DOG

Previous Serial Number: 4.15

Principal Investigator: Stephen Potkay

Other Investigators: Willard M. Dagget, Jr., NHI (Previously)
Joseph P. Gilmore, NHI

Cooperating Units: Laboratory of Cardiovascular Physiology, NHI

Man Years: (computed for the 12 month period)
Total: Less than .05

Project Description:

Objective: To determine whether urine flow during water diuresis is influenced by vagal afferents other than those associated with the heart.

Methods Employed: Water diuresis was established in the chronic cardiac denervated dog, a preparation in which all extrinsic cardiac nerves are removed. This was followed by bilateral cervical vagotomy. Parameters of renal function measured were: osmolal clearance, sodium, potassium and calcium excretion, and urine flow.

Major Findings: Cervical vagotomy decreased urine flow and free water clearance independently of changes in sodium, potassium, and calcium excretion, and osmolal clearance. The mechanism response is probably located within the chest and may be pulmonary in origin since all cardiac nerves were removed in the preparation used.

Significance: These findings give further information regarding mechanisms regulating body fluid volume.

Proposed Course of Project: Completed. A paper is being prepared for publication.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-13
1. Laboratory Aids Branch
2. Animal Hospital Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: USE OF SYNTHETIC MATERIALS IN REPAIR OF RUPTURED CANINE ANTERIOR CRUCIATE LIGAMENT

Previous Serial Number: None

Principal Investigator: Thomas P. Cameron

Other Investigators: Calvin E. Sevy

Cooperating Units: None

Man Years (computed for the 12 month period)

Total:	.05
Professional	.05
Other:	.00

Project Description:

Objective: To determine a simple method, using synthetic materials, in the repair of ruptured cruciate ligaments. In experimental animals the stifle joint is invaded and the ligament removed.

Methods Employed: Strips of various Teflon fabrics are attached to plastic buttons supplied by the Biomedical Engineering and Instrumentation Branch. The buttons are milled of an inert plastic, KEL-F. The surgical procedure previously utilized a strip of the patient's thigh connective tissue, the Tensor fasiae latae. This strip was hard to prepare, shredded easily in manipulations, and could be accidentally torn or cut.

Major Findings: The KEL-F button forms a secure proximal anchorage on the lateral aspect of the femoral condyles. The fabric is easily passed with an alligator forcep through holes drilled in the condyle and out to the medial aspect of the tibial crest where it is secured. With no fear of tearing, the fabric ligament can be sutured distally under firm tension. Animals will use the leg again for locomotion in three to seven days after surgery.

Significance: The rupture of this ligament is commonly diagnosed by veterinarians and must be surgically repaired. Although Teflon fabric has been used before to replace the ligament, this project introduces the idea of an improved proximal anchorage. In addition, three different Teflon weaves are being evaluated to determine differences between them.

Proposed Course of Project: Continuation. A manuscript will be prepared.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-14
1. Laboratory Aids Branch
2. Animal Hospital Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: EVALUATION OF RADIATION THERAPY ON CANINE MALIGNANT LYMPHOMA

Previous Serial Number: None

Principal Investigator: Ralph E. Johnson, NCI

Other Investigators: Thomas P. Cameron
Roy Kinard

Cooperating Units: Radiation Branch, NCI
Comparative Pathology Section, LAB

Man Years (computed for the 12 month period)
Total: .19
Professional: .04
Other: .15

Project Description:

Objective: To determine the effect of fractionated whole body radiation on spontaneous cases of canine malignant lymphoma or lymphosarcoma.

Methods Employed: Animals are referred by area veterinary practitioners. Blood samples, femoral bone marrow samples and lymph node biopsy are used to confirm diagnosis. Initially, fractionated doses were compared with single massive doses and sham treatments for efficacy. After fractionated treatment was proven conclusively to be best, protocol was changed to determine optimal treatment with varying intensities and frequencies of fractionated radiation. Efficacy is rated by reduction of total tumor mass, total body weight changes, and effect upon the solid blood components.

Major Findings: Fractionated radiation substantially reduced tumor size with minimal side effects on the patient. While untreated cases terminate in a four to six week period after diagnosis, judicious use of this regime has kept patients in total remission for up to five months.

Significance: The findings of this work can be applied to veterinary and human therapy. Use of radiation in fractionated carefully plotted increments might keep patients in indefinite remission alone or in conjunction with chemotherapy.

Proposed Course of Project: Continuation and eventual preparation of manuscript.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-15
1. Laboratory Aids Branch
2. Animal Hospital Section
3. NIH Animal Center

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: COLOR DESCRIPTION OF CATS

Previous Serial Number: None

Principal Investigator: Kenneth S. Brown, NIDR

Other Investigators: Calvin E. Sevy

Cooperating Units: Human Genetics Branch, NIDR

Man Years (computed for the 12 month period)
Total: .06
Professional: .04
Other: .02

Project Description:

Objective: To devise and utilize a code system to record the coat color and eye color of cats. To utilize this system and to enumerate the cats having different coat and eye characteristics as they are received at the NIH Animal Center.

Methods Employed: Each cat received is examined by NIHAC personnel and the coat and eye color recorded according to a chart. The classification is recorded on the individual record card of each animal then transferred to a data sheet each quarter.

Major Findings: Data have not been analyzed as yet,

Significance: There is a correlation between heterochromia and deafness in the cat as well as other animals. This system allows the investigator to record and analyze the coat color and eye color in large numbers of animals without lengthy word description. This system could also be used to discover other statistical relationships between disease experience and mortality or other descriptive traits.

Proposed Course of Project: Continuation.

Honors and Awards: None

Publications: None

Serial No. DRS-LAB-16

1. Laboratory Aids Branch
2. Comparative Pathology Section
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1965 through June 30, 1966

Project Title: ACUTE RESPIRATORY DISEASE IN PROCURED ADULT DOGS HELD FOR QUARANTINE AT THE NATIONAL INSTITUTES OF HEALTH ANIMAL CENTER (NIHAC)

Previous Serial Number: None

Principal Investigator: James R. Ganaway

Other Investigators: Thomas D. Moore
Anton M. Allen
Thomas P. Cameron

Cooperating Units: National Institutes of Health Animal Center

Man Years (computed for the 12 month period)

Total:	3.4
Professional:	.9
Other:	2.5

Project Description:

Objective: To isolate and identify the etiologic agent(s); to elucidate the disease syndrome and suggest means for prevention and/or control.

Methods Employed: Microbiology, immunology, epidemiology, and pathology.

Major Findings: As a result of isolation studies, controlled vaccine trials, and serological follow-up, Bordetella bronchiseptica is believed to play a major role in this respiratory disease syndrome. Perhaps far greater in importance, however, is the effect of stresses to which these dogs are subjected, starting from the time they are first "earmarked" for procurement until they are issued to the Institutes. The overly stressed animal with a much lowered resistance, in addition to representing fertile ground for opportunistic microbiota, responds poorly to medical treatment. If these conclusions and concepts are tenable and acceptable, we may then understand the failures of vigorous attempts of the past to cope with this costly and seemingly invincible problem. The best animal for research is the animal specifically raised for research. Implementation of such a concept would provide healthy dogs of uniform quality. As an interim measure, pertinent recommendations were submitted to NIHAC.

Significance: Acute respiratory disease occuring in 70% of the procured dogs within 10 to 20 days after receipt at NIHAC is characterized initially by purulent ocular and nasal discharges, tracheobronchitis accompanied by coughing, gagging and anorexia; later by dyspnea and bronchopneumonia terminating in death (approximately 5%). Such a syndrome has been observed in procured dogs at NIH for the past 10 years. A conservative estimate of the actual dollar cost of this syndrome to NIH is $40,000 yearly. In addition, as a result of the protracted illness (20 to 30 days), an undesireable specimen is sometimes issued to the Institutes.

Proposed Course of Project: Continuation.

Honors and Awards: None

Publications: None

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